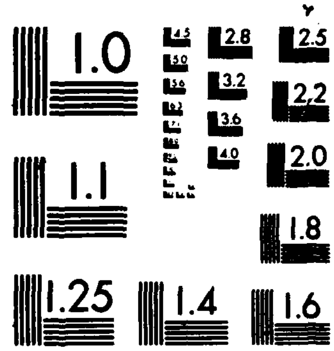
AD-A182 716   THE SYNTHESIS AND STUDY OF AZOLE CARBOXAMIDE NUCLEOSIDES AS AGENTS ACTIVE AGAINST RNA VIRUSES(U) BRIGHAM YOUNG UNIV PROVO UT   R K ROBINS ET AL.   1/1

UNCLASSIFIED   15 SEP 86 DAMD17-79-C-9046   F/G 6/15   NL

END
8-87
DTIC



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

(12)

AD-A182 716

AD ____________

THE SYNTHESIS AND STUDY OF AZOLE CARBOXAMIDE NUCLEOSIDES AS AGENTS ACTIVE AGAINST RNA VIRUSES

Annual/Final Report

Roland K. Robins, Ph.D.
Ganapathi R. Revankar, Ph.D.

September 15, 1986

Supported By
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-79-C-9046

Brigham Young University, Provo, Utah 84602

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188
Exp. Date Jun 30, 1986

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY 2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution unlimited
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Brigham Young University 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) Provo, UT 84602	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER Contract No. DAMD17-79-C-9046
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62770A; PROJECT NO. 3M1-62770A8[illegible]; TASK NO. AH; WORK UNIT ACCESSION NO 355

11. TITLE (Include Security Classification)
The Synthesis and Study of Azole Carboxamide Nucleosides as Agents Active Against RNA Viruses

12. PERSONAL AUTHOR(S)
Roland K. Robins, Ph.D., Ganapathi R. Revankar, Ph.D.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual/Final*	FROM 9/1/84 TO 8/31/85	September 15, 1986	84

16. SUPPLEMENTARY NOTATION
* Final for the period April 1, 1979 - August 31, 1985

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	13	
07	03	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
A number of 1,2,4-triazole, thiazole, pyrrole, pyrazole, purine, pyrrolo[2,3-d]pyrimidine and pyrimido[5,4-d]pyrimidine nucleosides have been prepared as potential anti-RNA viral agents.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

In an effort to prepare compounds with potential antiviral activity against RNA viruses having substantial implications from a global eipdemiological standpoint, a number of azole heterocycles and the corresponding nucleosides structurally related to ribavirin have been synthesized. 1,2,4-Triazole, thiazole, pyrrole, pyrazole, purine, pyrrolo[2,3-d]pyrimidine and pyrimido[5,4-d]pyrimidine ring systems have particularly been selected. New and improved synthetic procedures have been developed during the course of this study. The compounds th.s synthesized in gram quantities were tested at the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, against RVF, VEE, PICH, YF, SF, VSV and KHF viruses in vitro and in vivo. Keywords: [illegible]; Machupo virus; [illegible]

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Mrs. Virginia Miller	22b. TELEPHONE (Include Area Code) 301/663-7325 22c. OFFICE SYMBOL SGRD-RMI-S

DD FORM 1473, 84 MAR   83 APR edition may be used until exhausted
All other editions are obsolete
SECURITY CLASSIFICATION OF THIS PAGE

SUMMARY

Early studies with ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) against a broad-spectrum of viruses indicated it to be significantly active against a wide variety of both DNA and RNA viruses. Ribavirin has since been found effective clinically against several RNA virus diseases and has recently been approved by the FDA for use against respiratory syncytial virus infection in man.

Ribavirin remains to date the single most promising broad-spectrum antiviral agent, active against most of the major disease viruses of RNA type. Ribavirin is very effective against Rift Valley fever, Lassa, Machupo, Dengue, Pichinde, Hantaan and Retroviruses (e.g. HTLV-III) in vivo. The potent activity of ribavirin against Lassa fever in subhuman primate models is an indication of the potential human use of synthetic antiviral agents against virulent tropical RNA viral diseases. The success of ribavirin as a broad-spectrum antiviral agent has stimulated a great deal of effort toward the chemical synthesis of nucleosides of other azole heterocycles.

During the past year, a number of 1,2,4-triazole, thiazole, pyrrole, pyrazole, purine, pyrrolo[2,3-d]pyrimidine and pyrimido[5,4-d]pyrimidine nucleosides, and certain heterocycles have been prepared in sufficient quantity and submitted to Chemistry Handling and Data Analysis Branch, Division of Experimental Therapeutics, Department of Medicinal Chemistry, Walter Reed Army Institute of Research, Washington, D. C. for antiviral evaluation in both in vitro and in vivo.

TABLE OF CONTENTS

I. INTRODUCTION

Each year viruses cause over one-billion infections in the United States alone. In man, viral infections are responsible for some 60% of all the episodes of illness. About five-billion man days are lost each year in this country because of virus diseases. No other category of diseases approaches this total in terms of human disability. Man suffers approximately seven viral upper respiratory infections each year. A problem of viral diseases is that there are a considerable number of different viral strains which can cause infection. For example, over a hundred strains of rhinovirus, more than thirty adenovirus strains and over sixty coxsackie and echovirus strains are known. It is virtually impossible or impractical to produce a vaccine active against more than one strain and one type of virus. Therefore, vaccination may not be the answer.

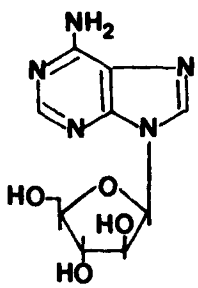
The major failure of the past several decades with regard to the development of successful chemotherapeutic agents against virus infection has been the close relationship that exists between the multiplying virus and the host cells. For most aspects of its growth, a virus depends upon the metabolism of its host cells and the majority of viral inhibitors act against cellular processes and thus are toxic for normal cells. However, the fruits of research during the last two decades heralded a new era of modern antiviral therapy.

A modern approach to viral chemotherapy as summarized by Professor Seymour Cohen[1] is as follows:

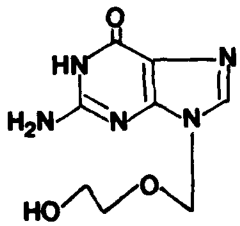
> That almost all viruses carry genes for the synthesis of new metabolic machinery was demonstrated in the last two decades. Because virus infected cells contain unique enzymes and proteins essential for virus reproduction, it should be possible to inhibit virus diseases specifically.

Today, we have overwhelming evidence that specific antiviral substances do exist which significantly suppress viral growth in concentrations which do not interfere with the basic cell functions.[2]

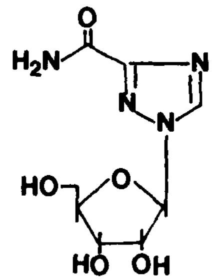
Progress made in the syntheses and development of antiviral agents has recently been reviewed.[3-7] Although the development in the area of viral chemotherapy was rather slow in the beginning, substantial progress has been made in recent years. The FDA approval of 9-β-D-arabinofuranosyladenine (ara-A, trade name Vidarabine) and 9-(2-hydroxyethoxymethyl)guanine (acyclovir) for use against herpes (DNA virus) infection, and 1-β-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin or virazole) against respiratory syncytial virus is indeed a major step forward. Ribavirin remains



ara-A



acyclovir



ribavirin

to date the single most promising broad-spectrum antiviral agent, active against most of the major disease viruses of both DNA and RNA type[8] and no ribavirin-resistant virus strains have been demonstrated *in vitro.*

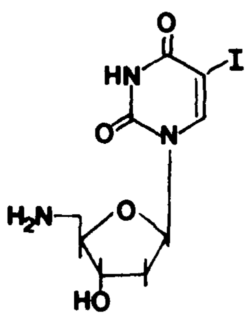
Ribavirin is a very specific nucleoside with amazingly stringent structural requirements for broad-spectrum antiviral activity and these structural requirements are regarded as necessary to transport the drug into cells. The relationship of antiviral efficacy and structure of ribavirin has recently been reviewed in detail by Harris and Robins,[9] and by Sidwell, Revankar and Robins.[10] The success of ribavirin as a broad-spectrum antiviral agent has stimulated a great deal of effort toward the chemical synthesis and antiviral screening of a number of nucleosides of azole heterocycles. During the last twelve months we continued our synthetic program designed to provide the selected ribavirin derivatives and related azole carboxamide nucleosides. Forty such compounds were prepared and submitted to Chemistry Handling and Data Analysis Branch, Division of Experimental Therapeutics, Department of Medicinal Chemistry, Walter Reed Army Institute of Research, Washington, D.C. for antiviral evaluation. The progress made in the synthetic aspect may be divided into four major categories:

1. Synthesis of Certain 5′-Substituted Derivatives of Ribavirin and Tiazofurin
2. Direct Glycosylation of Preformed Fully Aromatic Pyrroles
3. Synthesis of 4-Substituted-1-β-D-ribofuranosyl-3-hydroxypyrazoles Structurally Related to Pyrazofurin
4. Synthesis of 4-Amino-8-(β-D-ribofuranosylamino)pyrmido[5,4-d]pyrimidine and Other Miscellaneous Compounds

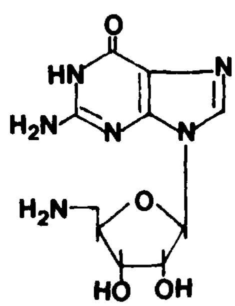
II. CHEMISTRY AND DISCUSSION

1. Synthesis of Certain 5′-Substituted Derivatives of Ribavirin and Tiazofurin

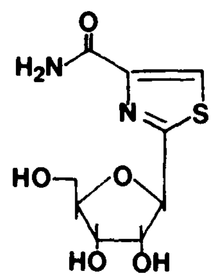
Efforts have been directed, in recent years, toward the synthesis of 5′-amino-5′-deoxypyrimidine and purine nucleosides that are more selective in their antiviral effects. The highly selective antiviral activity of



1



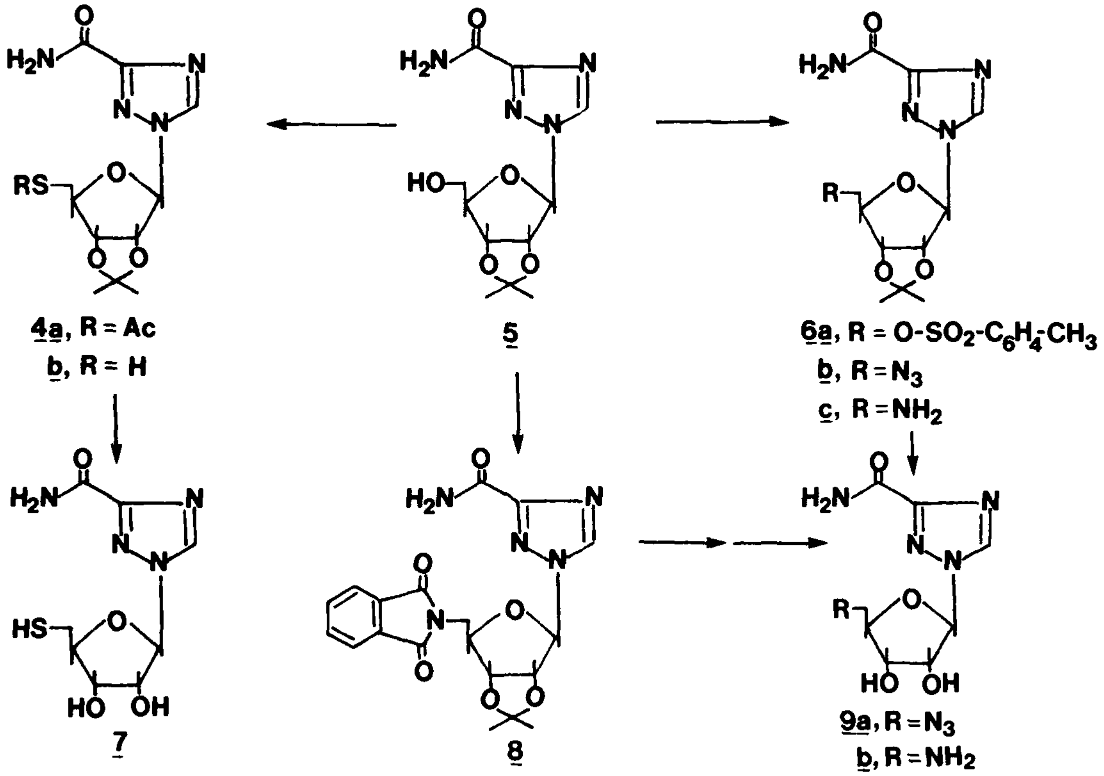
2



3
tiazofurin

5′-amino-2′,5′-dideoxy-5-iodouridine (1) against HSV *in vitro* has been well documented.[11,12] Although this 5′-amino-5′-deoxypyrimidine nucleoside is incorporated into both viral and cellular DNA, such incorporation is restricted to the infected cells only.[13,14] The increase of the antiviral therapeutic index of 5-trifluoromethyl-2′-deoxyuridine by a factor of 10 by replacement of the 5′-hydroxyl with an amino group is another convincing example.[15] 5′-Amino-5′-deoxyguanosine[16] (2) also produced a highly significant inhibition (>80%) of a number of RNA virus replication at concentrations as low as 6.4 μg/ml. No cytotoxicity was observed with 2 even at concentrations as high as 1000 μg/ml. Compound 2, which exhibited significant inhibition of MLV replication in vitro at a concentration of 0.64 μg/ml, gave a "selectivity ratio" of >1500.[17] The structure of ribavirin as noted by single crystal X-ray studies,[18] is strikingly similar to that of guanosine with the carbonyl oxygen and the amide nitrogen occupying stereochemically similar positions to the carbonyl oxygen (O^6) and the amide ring nitrogen (N^1) in guanosine. In view of these findings we have now prepared the 5′-amino-5′-deoxy derivatives of ribavirin, as well as the synthetic oncolytic C-nucleoside tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, 3). Tiazofurin, synthesized and reported simultaneously from our laboratory[19] and by Fuertes et al.,[20] is a promising antitumor agent[21-23] currently undergoing Phase II clinical trials. Tiazofurin shows significant antiviral activity in vitro[19] and potent activity against several murine tumors, including Lewis lung carcinoma.[24-26]

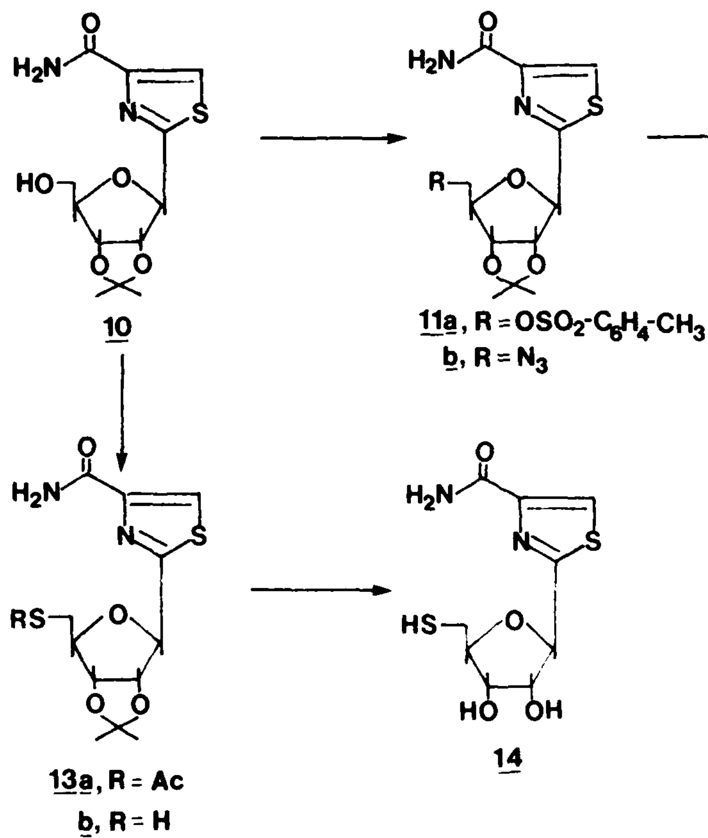
The synthesis of 5′-amino-5′-deoxyribavirin (9b) via the intermediate 5′-iodo-5′-deoxyribavirin is unsatisfactory due to poor yields.[27] Further studies in our laboratory resulted in the successful synthesis of 9b by two different routes. Tosylation of 2′,3′-O-isopropylidene ribavirin[28] (5) with p-toluenesulfonyl chloride in dry pyridine at 0-4°C[29] gave 1-(2,3-O-isopropylidene-5-O-p-tolylsulfonyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (6a, BL-00334) in 89% yield. The 5′-O-p-tolylsulfonyl group of 6a was selectively displaced by the 5′-azido function (6b, BL-00325) by



reacting with sodium azide in dry DMF at 85-90°C for 10 hr according to the general procedure of Horwitz et al.[30] Deisopropylidenation of 6b with 80% acetic acid gave 9a (BL-07333), which on further catalytic (Pd/C) hydrogenation[31] in EtOH-H_2O (1:1) at room temperature and 37 psi of hydrogen pressure afforded the desired 9b in 70% yield. Alternatively, when compound 5 was allowed to react with diethyl azodicarboxylate, triphenylphosphine and phthalimide in THF at room temperature overnight,[32] 1-(2,3-O-isopropylidene-5-deoxy-5-phthaloylamino-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (8) was obtained in a 60% yield. On treatment of 8 with methanolic n-butylamine under reflux for 12 hr, afforded the corresponding 5'-amino-5'-deoxy derivative 1-(2,3-O-isopropylidene-5-deoxy-5-amino-β-D-ribofuranosyl)1,2,4-triazole-3-carboxamide (6c, BL-04181), which on subsequent deisopropylidenation provided yet another route to 9b.

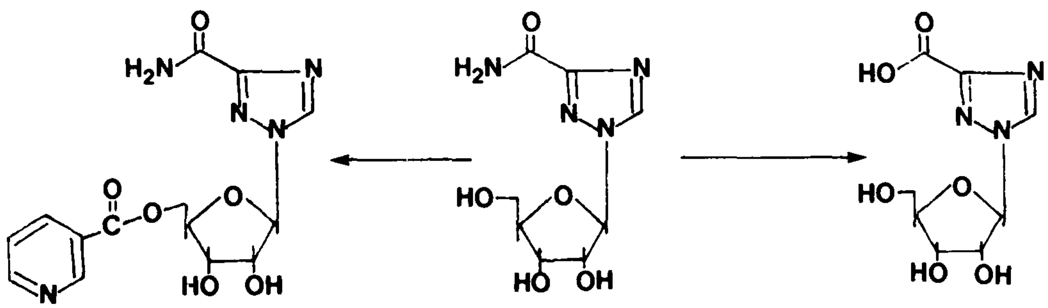
A highly efficient method for the conversion of alcohols to thiolesters and thiols has recently been described.[33] Application of this procedure to 2′,3′-O-isopropylidene ribavirin (5) by treatment with diisopropyl azodicarboxylate, triphenylphosphine and thiolacetic acid in THF at 0-5°C readily gave 1-(2,3-O-isopropylidene-5-deoxy-5-acetylthio-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (4a) in excellent yield. That the carbamoyl function on the aglycon remained unacylated was confirmed by its ^{1}H NMR spectrum, which revealed the carbamoyl protons at δ 7.68 and 7.88 ppm. The free thiol (4b, BL-04190) was obtained by saponification (NaOMe) of 4a. Acid catalyzed deisopropylidenation of 4b provided 1-(5-deoxy-5-thio-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (7, BL-04207).

Studies were extended to provide several 5′-substituted tiazofurin derivatives. Treatment of 2-(2,3-O-isopropylidene-β-D-ribofuranosyl)thia-



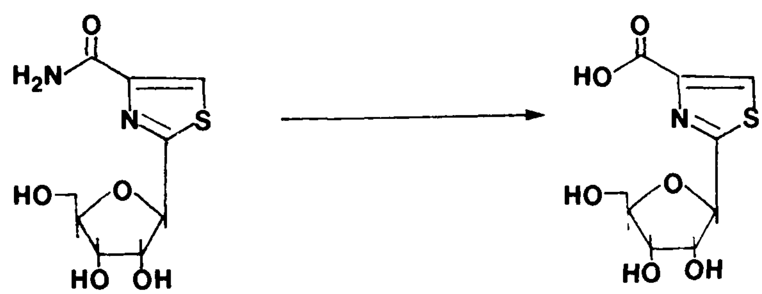
zole-4-carboxamide[20] (10) with triphenylphosphine, diisopropyl azodicarboxylate and thiolacetic acid in dry THF gave an 84% yield of the corresponding 5′-deoxy-5′-acetylthio derivative 2-(5-deoxy-5-acetylthio-2,3-di-O-isopropylidene-β-D-ribofuranosyl)thiazole-4-carboxamide (13a, BL-07351). Saponification of 13a with sodium methoxide in MeOH afforded 13b, which on acid catalyzed deisopropylidenation furnished 5′-deoxy-5′-thiotiazofurin [2-(5-deoxy-5-thio-β-D-ribofuranosyl)thiazole-4-carboxamide, 14] in a 84% yield.

5′-Amino-5′-deoxytiazofurin (15) was prepared by employing the procedure that was used to obtain 5′-amino-5′-deoxyribavirin (9b). Thus, tosylation of 10 with p-toluenesulfonyl chloride in dry pyridine gave 2-(2,3-O-isopropylidene-5-O-p-tolylsulfonyl-β-D-ribofuranosyl)thiazole-4-carboxamide (11a) in 69.3% yield, which on treatment with lithium azide in DMF at 85-90°C for 20 hr furnished the corresponding 5′-azido-5′-deoxy derivative (11b). Removal of the 2′,3′-O-isopropylidene blocking groups of 11b with hot 80% acetic acid gave 2-(5-deoxy-5-azido-β-D-ribofuranosyl)thiazole-4-carboxamide (12, BL-07342). Hydrogenation of 12 in 50% aqueous ethanol in the presence of Pd/C to obtain the desired 2-(5-amino-5-deoxy-β-D-ribofuranosyl)thiazole-4-carboxamide (15) is in progress.



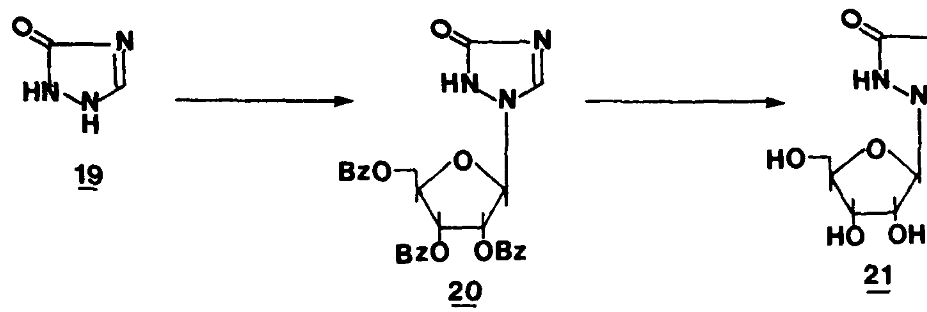
16 ribavirin 17

Direct acylation of ribavirin was accomplished by adding 1.1 molar equivalent of nicotinoyl chloride to a solution of ribavirin in 1:1 mixture of pyridine:N,N-dimethylformamide. This solvent mixture was found to greatly facilitate the selectivity of the acylation of the primary hydroxyl group over either of the secondary hydroxyl groups.[34] This could be a consequence of having the acylating agent in a charged species (like N-acylpyridinium chloride) in an aprotic, polar solvent, such as DMF.[35,36] 1-(5-O-Nicotinoyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (16, BK-98937) was isolated in 44% yield as crystalline solid after column chromatography over silica gel to separate minor, peracylated contaminants and unreacted ribavirin. That the carbamoyl function on the aglycon remained unacylated was confirmed by its ^1H NMR spectrum in Me_2SO-d_6, that showed the carbamoyl protons at δ 7.60 and 7.80 ppm. The ^1H NMR spectrum also revealed an expected downfield shift[34] for the 5′-methylene function that was consistent with 5′-O-acylation of ribavirin. Saponification of ribavirin with 6N NaOH readily gave 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxylic acid (17, BL-00281). A similar treatment of tiazofurin (3) with 6N NaOH furnished 2-β-D-ribofuranosylthiazole-4-carboxylic acid (18, BL-00272).



3 18

Direct glycosylation of the nonsilylated 1,2,4-triazol-3(2H)-one (19) with the blocked benzoyl sugar in the presence of the catalyst $BF_3.OEt_2$ in a boiling polar aprotic solvent such as nitromethane gave a nucleoside product, identified as 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,2,4-triazol-3(2H)-one (20, BL-00307). No formation of other isomeric nucleosides was observed. Debenzoylation of 20 with sodium methoxide in methanol readily gave 1-β-D-ribofuranosyl-1,2,4-triazol-3(2H)-one (21, BL-00290).

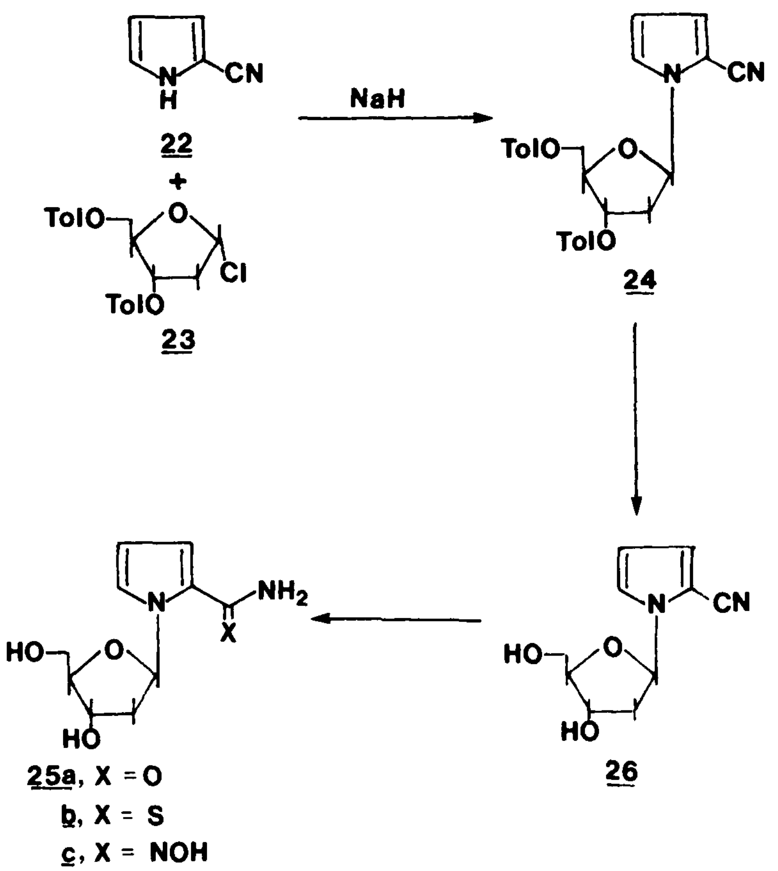


2. <u>Direct Glycosylation of Preformed Fully Aromatic Pyrroles</u>

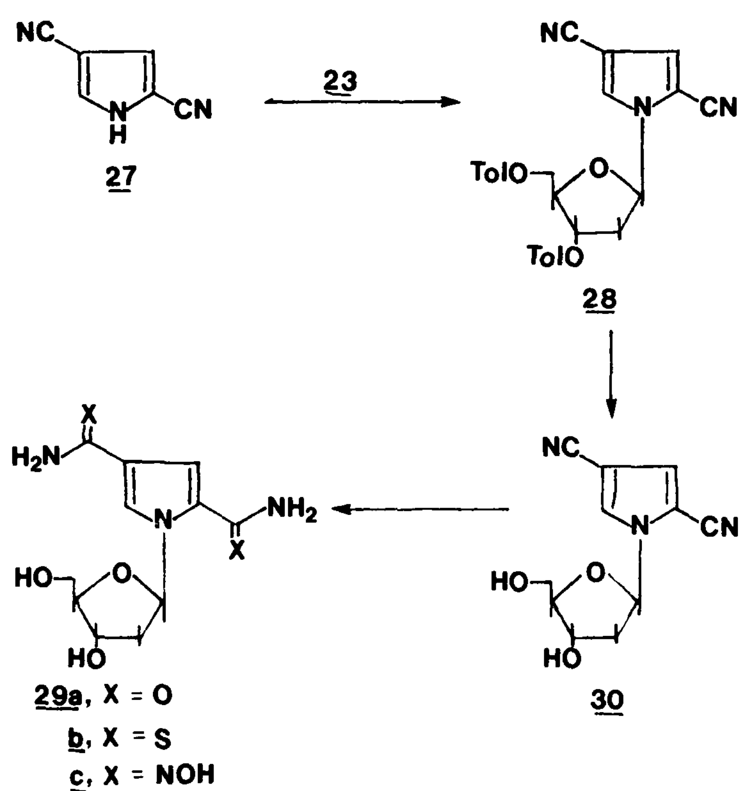
The stereospecific synthesis of 2′-deoxyribofuranosyl nucleosides with β-anomeric configuration has been a part of this research program. Prior glycosylation procedures introducing the 2-deoxy-β-D-ribofuranosyl (2-deoxy-β-D-*erythro*-pentofuranosyl) moiety into an azole heterocycle reported from our laboratory[37-40] and by others[41-44] invariably suffered from the need to separate regioisomers and anomers at some stage of the synthetic sequence. In view of these difficulties, a four-step deoxygenation procedure using phenoxythiocarbonylation[45-47] or imidazolylthiocarbonylation[48,49] of the 2′-hydroxyl group of the corresponding 3′,5′-diprotected β-D-ribonucleoside has been developed to provide the requisite 2′-deoxynucleoside. These latter procedures however, require the availability of the preformed ribonucleoside. Although the synthesis of a number of analogs of 2′-deoxyadenosine by an enzymatic procedure has been reported,[50] this approach is not generally applicable to certain pyrrolopyrimidine nucleosides.[51] We have recently employed a sodium salt glycosylation procedure[52-57] for the synthesis of several ribo and 2′-deoxyribofuranosyl derivatives of certain heterocyclic ring systems. Use of this stereospecific, single-phase sodium salt glycosylation procedure for the synthesis of pyrrole nucleosides has now been found to be remarkably successful. Further ring annulation of certain of these pyrrole nucleosides provided a route to the synthesis of 2′-deoxytoyocamycin derivatives.

A search of the literature revealed that only a few pyrrole N-nucleosides have been reported in the literature,[58-60] which utilized partially hydrogenated pyrroles in the glycosylation reaction employing the "indoline-indole" method.[61] Subsequent photodehydrogenation of Δ^3-pyrroline nucleoside intermediates afforded the pyrrole nucleosides. However, the present synthetic pathway involves the direct attachment of a glycon moiety to a preformed fully aromatic pyrrole derivative.

In the present work we elected to use pyrrole-2-carbonitrile[62] (22) as one of the aglycons for glycosylation studies. The sodium salt of 22, generated *in situ* by NaH in anhydrous acetonitrile, was treated with 1-chloro-2-deoxy-3,5-di-O-p-toluoyl-α-D-*erythro*-pentofuranose[63] (23) at ambient tem-

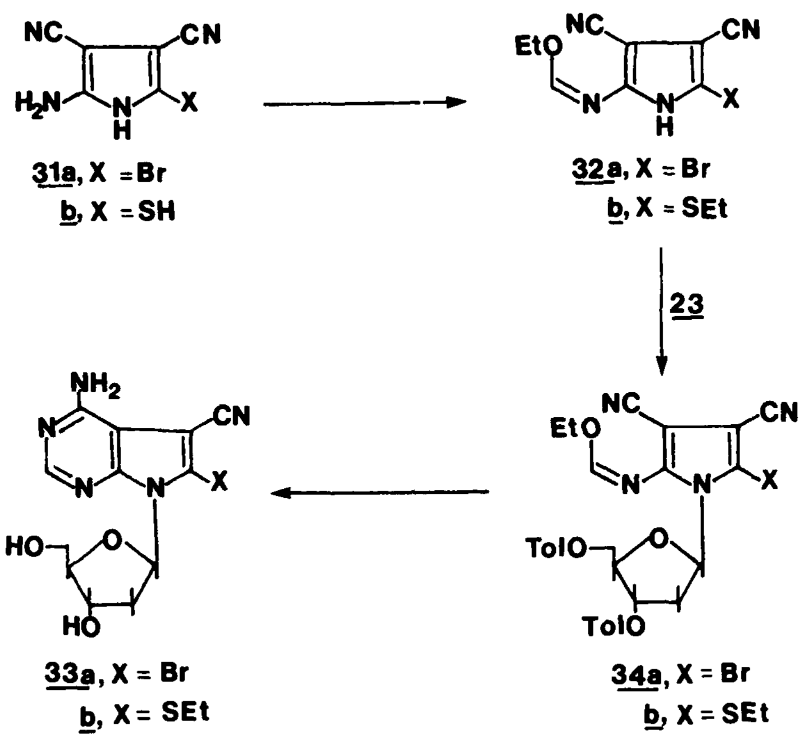


perature. A clean reaction was observed, and the desired 1-(2-deoxy-3,5-di-O-p-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrole-2-carbonitrile (24, BK-98900) was isolated in 67% yield. No formation of the α-anomer was detected in this reaction. Deprotection of the blocking groups of the glycon moiety of 24 was accomplished by the treatment with methanolic ammonia at room temperature to yield 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrole-2-carbonitrile (26, BL-00361), in which the nitrile function was available for further transformation reactions. The presence of the nitrile function in 26 was evident as confirmed by the IR spectrum, which revealed a sharp absorption band at 2220 cm^{-1}. Treatment of 26 with NH_4OH/H_2O_2 solution, and purification of the reaction product by chromatography on silica gel furnished 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrole-2-carboxamide (25a, BL-04145) in good yield. Reaction of 26 with H_2S in pyridine containing Et_3N at room temperature gave the corresponding thiocarboxamide derivative 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrole-2-thiocarboxamide (25b, BL-04154). When 26 was allowed to react with free NH_2OH in ethanol at reflux temperature, 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrole-2-amidoxime (25c, BL-07360) was formed in 90% yield.



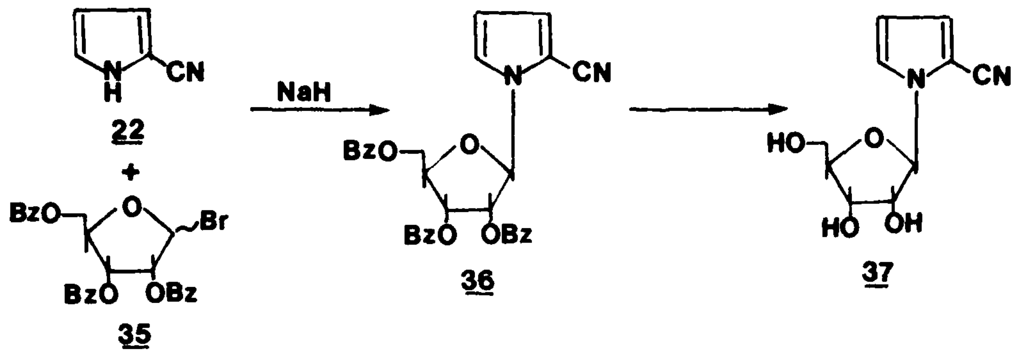
The other heterocycle that was employed for glycosylation studies was pyrrole-2,4-dicarbonitrile[62] (27, BK-98884). Reaction of the protected halogenose 23 with the sodium salt of 27 gave a 68% yield of 1-(2-deoxy-3,5-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)pyrrole-2,4-dicarbonitrile (28, BK-98893). As in the case of 24, no formation of the α-anomer of 28 in this reaction was observed. Deprotection of the blocking groups of the glycon moiety of 28 was accomplished by the treatment with methanolic ammonia at room temperature to yield 1-(2-deoxy-β-D-erythro-pentofuranosyl)pyrrole-2,4-dicarbonitrile (30) in 74% yield. Treatment of 30 with NH_4OH/H_2O_2 solution, and purification of the reaction product by chromatography on silica gel furnished 1-(2-deoxy-β-D-erythro-pentofuranosyl)-pyrrole-2,4-dicarboxamide (29a, BL-00352) in 83% yield. Reaction of 30 with H_2S in pyridine gave 1-(2-deoxy-β-D-erythro-pentofuranosyl)pyrrole-2,4-bisthiocarboxamide (29b, BL-07388), which was isolated in 62% yield. When 30 was allowed to react with free NH_2OH in EtOH at reflux temperature, 1-(2-deoxy-β-D-erythro-pentofuranosyl)pyrrole-2,4-bisamidoxime (29c, BL-07397) was formed and was isolated in 70% yield.

Application of this stereospecific sodium salt glycosylation procedure for the synthesis of fully aromatic pyrrole nucleosides and further ring annulation of certain of these pyrrole nucleosides provided a convenient route to the synthesis of 2′-deoxytoyocamycin derivatives. For the synthesis of such pyrrolo[2,3-d]pyrimidine nucleosides, 2-amino-5-bromopyrrole-3,4-dicarbonitrile (31a, BK-98875) served as a useful starting material. Compound 31a was prepared as reported in the literature.[64] The protection of the amino group of 31a was effected in 90% yield by the treatment with diethoxymethylacetate in acetonitrile at reflux temperature to give 2-ethoxymethyleneamino-5-bromopyrrole-3,4-dicarbonitrile (32a) in excellent yield. Coupling of 32a with 23 in acetonitrile furnished a 75% yield of 5-bromo-2-ethoxymethylenamino-1-(2-deoxy-3,5-di-O-p-toluoyl-β-D-erythro-

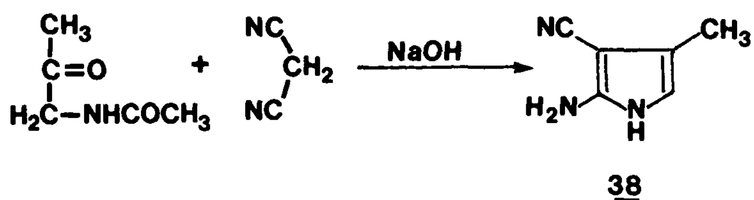


pentofuranosyl)pyrrole-3,4-dicarbonitrile (34a, BL-07379), which cleanly cyclized to 4-amino-6-bromo-7-(2-deoxy-β-D-erythro-pentofuranosyl)pyrrolo-[2,3-d]pyrimidine-5-carbonitrile (33a) on treatment with methanolic ammonia at room temperature.

2-Amino-5-mercaptopyrrole-3,4-dicarbonitrile (31b, BL-00343) was also prepared as reported in the literature.[64] The protection of the amino group of 31b by the treatment with diethoxymethylacetate and subsequent ethylation with ethyl iodide gave the key intermediate 2-ethoxymethylene-amino-5-ethylthiopyrrole-3,4-dicarbonitrile (32b). Condensation of 32b with the halogenose 23 in CH_3CN afforded a good yield of 5-ethylthio-2-ethoxymethylenamino-1-(2-deoxy-3,5-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)pyrrole-3,4-dicarbonitrile (34b, BL-04163), which readily cyclized to 4-amino-6-ethylthio-1-(2-deoxy-β-D-erythro-pentofuranosyl)pyrrolo[2,3-d]-pyrimidine-5-carbonitrile (33b, BL-04172) when treated with methanolic ammonia.



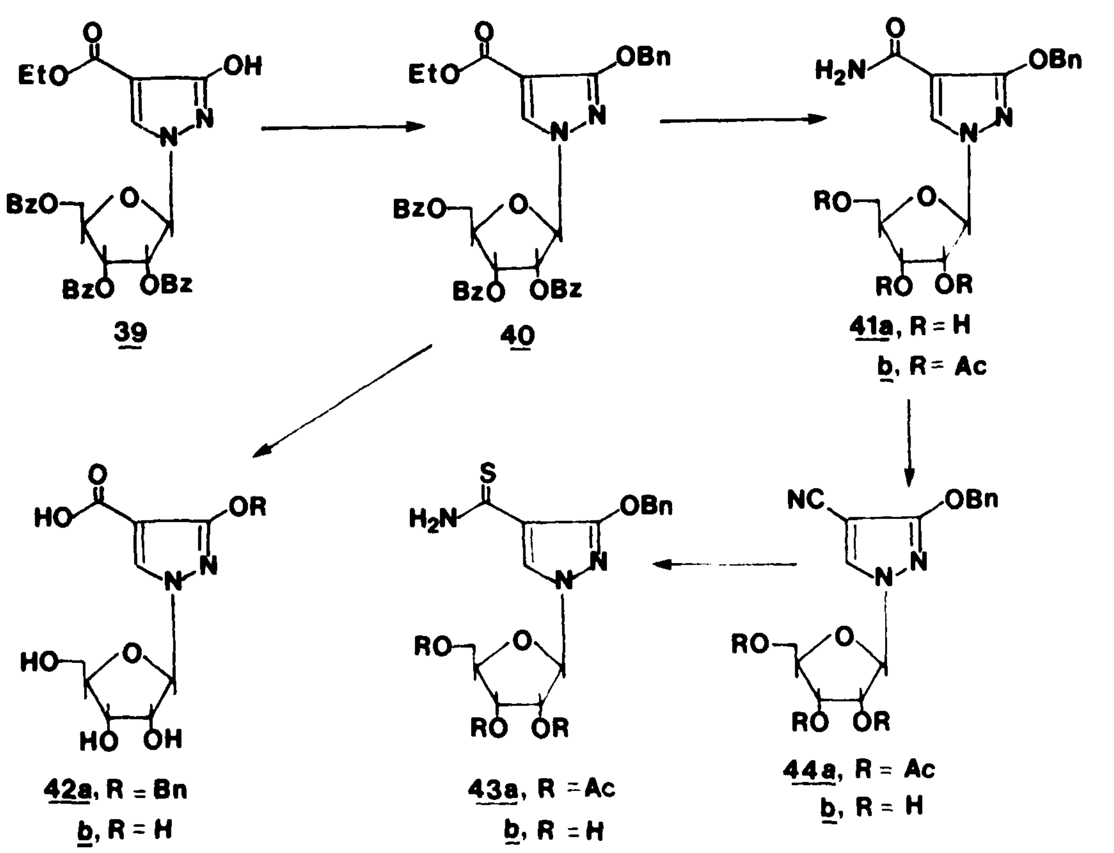
Pyrrole-2-carbonitrile (22) also served as a versatile starting material for ribosylation studies. 2,3,5-Tri-O-benzoyl-D-ribofuranosyl bromide (35) was freshly prepared from 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose according to the method of Fletcher and co-workers.[65] The sodium salt of 22, produced in situ by sodium hydride in dioxane, was treated with 35, and the reaction product was purified on a silica gel column and tentatively identified as 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrrole-2-carbonitrile (36, BL-00370). Studies are under progress to debenzoylate 36 with MeOH/NH_3 to obtain 1-β-D-ribofuranosylpyrrole-2-carbonitrile (37) and further functional group transformation of the carbonitrile group of 37.



During the course of these synthetic studies, preparation of 2-amino-4-methylpyrrole-3-carbonitrile (38, BK-98866) was also accomplished by condensing acetamidoacetone and malononitrile in the presence of NaOH according to the procedure of Wamhoff and Wehling.[66]

3. Synthesis of 4-Substituted-1-β-D-ribofuranosyl-3-hydroxypyrazoles Structurally Related to Pyrazofurin

Pyrazofurin (4-hydroxy-3-β-D-ribofuranosylpyrazole-5-carboxamide) is a naturally occurring azolecarboxamide C-nucleoside antibiotic possessing significant antiviral activity in cell culture against a broad spectrum of RNA viruses[67-69] at concentrations as low as 0.01 μg/mL.[70] Although pyrazofurin has a high degree of selectivity in its antiviral effects and shows a rather broad safety margin in cell culture, the LD_{50} dose in mice is about 5 mg/kg/day.[70] De Clercq and Torrence[69] suggest that this unexpected toxicity is probably not associated with the structural features of the molecule responsible for the antiviral potency. However, the toxicity of pyrazofurin is such that it cannot be separated from its antiviral efficacy in animals.[71,72] In an effort to decrease the toxic properties of pyrazofurin and hopefully retain the antiviral potency, we have now synthesized certain N-nucleoside congeners of pyrazofurin.



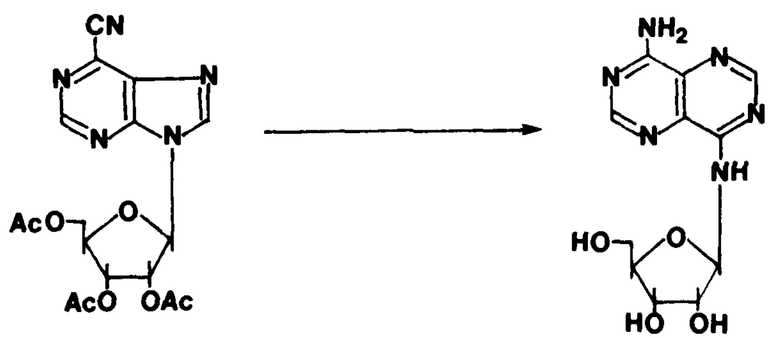
Glycosylation of the TMS derivative of ethyl 3(5)-hydroxypyrazole-4-carboxylate (BK-38388) with 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose in anhydrous CH_3CN in the presence of 1.4 molar equivalent of TMS-triflate at ambient temperature gave predominantly ethyl 3-hydroxy-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrazole-4-carboxylate (39). Benzylation of the sodium salt of 39, produced in situ by NaH in CH_3CN, with benzyl bromide gave ethyl 3-benzyloxy-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrazole-4-carboxylate (40) in excellent yield. Ammonolysis of 40 with $MeOH/NH_3$ furnished 3-benzyloxy-1-β-D-ribofuranosylpyrazole-4-carboxamide (41a) in over 80% yield. Acetylation of 41a with acetic anhydride in the presence of DMAP at ambient temperature gave 3-benzyloxy-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole-4-carboxamide (41b), which on dehydration with $POCl_3$ in the presence of N,N-diethylaniline at room temperature provided 3-benzyloxy-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole-4-carbonitrile (44a). Deacetylation of 44a with liquid NH_3 gave crystalline 3-benzyloxy-1-β-D-ribofuranosylpyrazole-4-carbonitrile (44b, BK-96675). Compound 44b revealed a strong C≡N stretching at 2210 cm^{-1} in the IR spectrum. Further treatment of 44a with H_2S in a pyridine solution at room temperature, and subsequent deacetylation of the reaction product (43a) with $MeOH/NH_3$ furnished 3-benzyloxy-1-β-D-ribofuranosylpyrazole-4-thiocarboxamide (43b, BK-96666). Attempted reductive clearage of the benzyl ether of either 43a or 43b with Pd/C, Na/NH_3[73] or sodium naphthalene[74] in THF resulted in an intractable reaction mixture from which the desired debenzylated product of 43b could not be isolated.

Saponification of the ester function of 40 by the treatment of 6N NaOH at room temperature gave 3-benzyloxy-1-β-D-ribofuranosylpyrazole-4-carboxylic acid (42a, BL-04127), which on catalytic hydrogenation readily provided 3-hydroxy-1-β-D-ribofuranosylpyrazole-4-carboxylic acid (42b, BL-04136).

The absolute structural assignment of 42a was made on the basis of single-crystal X-ray crystallographic studies. (See Appendix).

4. Synthesis of 4-Amino-8-(β-D-ribofuranosylamino)pyrimido[5,4-d]pyrimidine and Other Miscellaneous Compounds

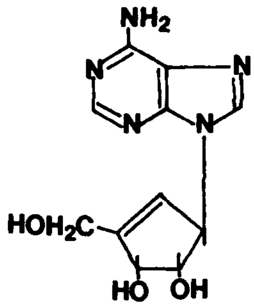
In view of the potent broad spectrum antiviral activity of 4-amino-8-(β-D-ribofuranosylamino)pyrimido[5,4-d]pyrimidine (46, BJ-76187) in cell culture,[75] and significant in vivo activity against Rift Valley fever virus[76], more of BJ-76187 has been prepared as reported.[75] Treatment of 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine-6-carbonitrile[77] (45) with a large excess of NH_4OH at room temperature gave the rearrangement product 46 in an 80% yield.



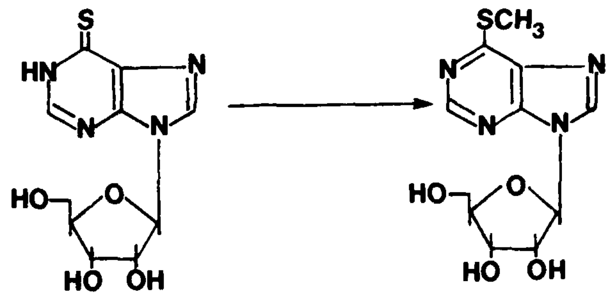
45

46

Neplanocins are a group of novel carbocyclic analogs of purine nucleosides which are isolated from the culture filtrate of Ampullarilla regularis A11079. These antibiotics exhibit potent antitumor properties. The culture filtrates of a fermentation broth produced by neplanocin A-producer CL-1018 was obtained from Warner-Lambert Pharmaceutical Research Division, Ann Arbor, Michigan. Isolation of the antibiotic neplanocin A was performed by the successive column chromatography on ion-exchange resin and charcoal, and by partition.[78] Neplanocin A (47, BL-04118) was isolated as the major product, and fully characterized.



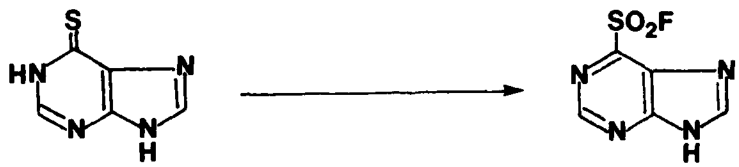
47



48 49

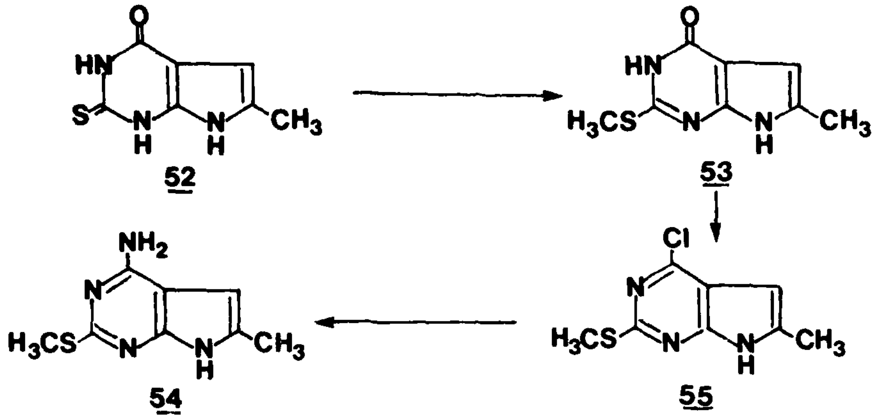
Methylation of 9-β-D-ribofuranosylpurine-6-thione[79] (48) with methyl iodide under alkaline conditions gave 6-methylthio-9-β-D-ribofuranosylpurine (49, BK-98928).

Several heterocyclic precursors, needed either for further ring annulation or for glycosylation studies, have been prepared. When purine-6-thiol (50) was suspended in a mixture of MeOH, aqueous hydrofluoric acid and KF and treated with chlorine gas near 0°C, purine-6-sulfonyl fluoride[80] (51, BL-00263) was formed in about 90% yield.

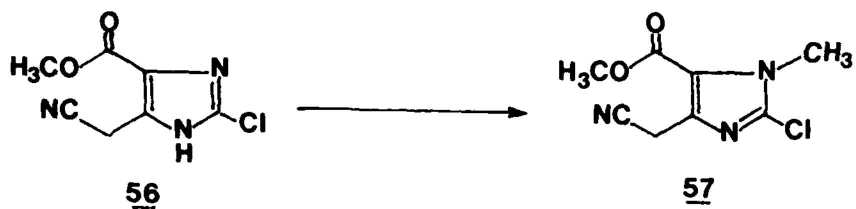


50 51

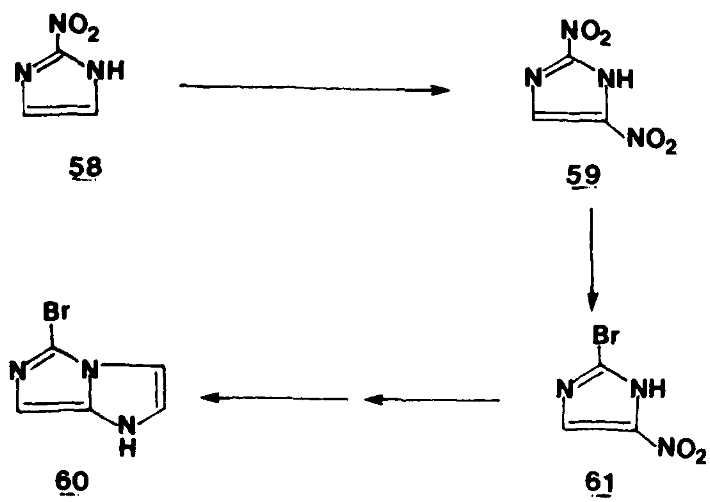
In an effort to prepare 4-amino-6-methyl-2-methylthiopyrrolo[2,3-d]-pyrimidine (54), the readily available 6-methyl-2-thiopyrrolo[2,3-d]pyrimidine-4(1H,3H)-dione[55] (52) was methylated with methyl iodide to obtain the corresponding 2-methylthio derivative (53). Chlorination of 53 with $POCl_3$ in the presence of N,N-dimethylaniline afforded 4-chloro-6-methyl-2-methylthiopyrrolo[2,3-d]pyrimidine (55). Ammonolysis of 55 with $MeOH/NH_3$ gave the desired 54 (BK-96693) in good yield.[55]



Methylation of methyl 2-chloro-5-cyanomethylimidazole-4-carboxylate[81] (56) with dimethyl sulfate under alkaline conditions gave a monomethylated product identified as methyl 2-chloro-5-cyanomethyl-3-methylimidazole-4-carboxylate (57, BK-96684).



In an attempt to prepare the azapentalene imidazo[1,5-a]imidazole, which will be employed for glycosylation studies, nitration of 2-nitro-



imidazole[82] (azomycin, 58) was considered. Thus nitration of 58 with 86% HNO_3 at elevated temperature gave 2,4(5)-dinitroimidazole[83] (59, BK-98919). Selective displacement of the nitro group in position 2 of 59 should give 2-bromo-4(5)-nitroimidazole (61), which on further ring annulation is expected to give the desired 5-bromoimidazo[1,5-a]imidazole (60) and these studies are in progress.

III. EXPERIMENTAL

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^1H NMR) spectra were determined at 89.6 MHz with a JEOL FX-90Q spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. The presence of solvent as indicated by elemental analysis was verified by ^1H NMR. Infrared spectra (IR) were obtained on a Beckman Acculab 2 spectrophotometer and ultraviolet spectra (UV; sh = shoulder) were recorded on a Cary Model 15 spectrophotometer. Elemental analyses were performed by Robertson Laboratory, Florham Park, N.J. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 plates (EM Reagents). E. Merck silica gel (230-400 mesh) was used for column chromatography. All solvents used were reagent grade. Detection of nucleoside components on TLC was by UV light and with 10% H_2SO_4 in MeOH spray followed by heating. Evaporations were carried out under reduced pressure with the bath temperature below 30°C.

1-(2,3-O-Isopropylidene-5-O-p-tolylsulfonyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (6a). A suspension of 2′,3′-O-isopropylidene ribavirin[28] (5, 2.84 g, 10 mmol) in anhydrous pyridine (35 mL) was cooled to 0°C in an ice bath, p-toluenesulfonyl chloride (2.10 g, 11 mmol) was added, and the mixture was stirred for 22 hr while being cooled in an ice bath.

The solution was concentrated to one-third of its original volume, and the resulting syrup was poured, with stirring, into ice-water (200 mL). The resulting gummy precipitate was extracted with EtOAc (200 mL), the organic phase was dried (Na_2SO_4) and evaporated to dryness. Crystallization of the residue from benzene gave colorless needles; yield 3.90 g (89%); mp 132-134°C.

<u>Chromatography:</u>	Absorbent - silica gel Solvent - CH_2Cl_2:MeOH, 9:1, v/v
<u>Infrared</u> (KBr):	Major bands - 780, 805, 860, 970, 1085, 1100, 1170 1210, 1270, 1350, 1450, 1590, 1680, 2980, 3120, 3180, 3350 and 3460 $cm.^{-1}$
^{1}<u>H NMR</u> (Me_2SO-$\underline{d}_6$):	δ 1.28 and 1.46 (2s, 6, $2C\underline{H}_3$), 2.40 (s, 1, $C\underline{H}_3$), 6.32 (d, 1, J = 0.5 Hz, $C_{1'}\underline{H}$), 7.32-7.80 (m, 6, aromatic protons and $CON\underline{H}_2$), 8.72 (s, 1, $C_5\underline{H}$).

		C	H	N	S
<u>Analysis:</u>	Calcd for $C_{18}H_{22}N_4O_7S$:	49.32	5.06	12.78	7.30
	Found:	49.32	5.09	13.07	7.47

<u>1-(2,3-O-Isopropylidene-5-azido-5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide</u> (<u>6b</u>). A solution of <u>6a</u> (4.38 g, 10 mmol) and sodium azide (1.95 g, 30 mmol) in dry DMF (150 mL) was stirred for 10 hr at 85-90°C and evaporated to dryness at 50°C. The residue was co-evaporated several times with EtOH and then triturated with cold water (150 mL). The solid that separated was collected by filtration and crystallized from MeOH to yield 2.70 g (87.4%) of <u>6b</u>; mp 184-186°C (dec.).

Chromatography: Absorbent - silica gel

Solvent - CH_2Cl_2:MeOH, 9:1, v/v

Infrared (KBr): Major bands - 760, 800, 840, 860, 980, 1005, 1030, 1070, 1090, 1160, 1180, 1210, 1250, 1280, 1340, 1380, 1460, 1600, 1710, 2100, 3000, 3320 and 3480 $cm.^{-1}$

Ultraviolet: λ_{max} (pH 7) 266 nm (ε 2,800);

λ_{max} (pH 11) 260 nm (ε 1,100).

^{1}H NMR (Me_2SO-d_6): δ 1.32 and 1.50 (2s, 6, $2CH_3$), 6.38 (d, 1, J = 1.8 Hz, $C_{1'}H$), 7.70 and 7.92 (2s, 2, $CONH_2$), 8.87 (s, 1, C_5H).

		C	H	N
Analysis:	Calcd for $C_{11}H_{15}N_7O_4$:	42.72	4.89	31.70
	Found:	42.56	4.79	31.47

1-(5-Deoxy-5-azido-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (9a). A suspension of compound 6b (6.18 g, 20 mmol) in 80% acetic acid (200 mL) was heated on a steam bath for 2 hr. A clear solution thus obtained was evaporated to dryness and the residue was purified on a silica gel column (2.5 x 40 cm) using $CHCl_3$:MeOH (6:1, v/v) as the eluent. Crystallization of the homogeneous product from MeOH gave 4.40 g (83.6%) of 9a; mp 124-125°C.

Chromatography: Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 9:1, v/v

Infrared (KBr): Major bands - 690, 750, 820, 850, 910, 960, 1020, 1060, 1125, 1180, 1215, 1270, 1440, 1480, 1610, 1650, 2100, 2920 and 3420 $cm.^{-1}$

^{1}H NMR (Me_2SO-d_6): δ 5.92 (d, 1, J = 2.0 Hz, $C_{1'}H$), 7.63 and 7.83 (2 br s, 2, $CONH_2$), 8.85 (s, 1, C_5H).

		C	H	N
Analysis:	Calcd for $C_8H_{11}N_7O_4$:	35.69	4.12	36.42
	Found:	35.82	3.99	36.20

1-(2,3-O-Isopropylidene-5-deoxy-5-phthaloylamino-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (8). To a cold (0-5°C) solution of triphenylphosphine (3.0 g, 12 mmol) and diisopropyl azodicarboxylate (2.50 g, 12 mmol) in anhydrous THF (50 mL) were added 5 (2.84 g, 10 mmol) and phthalimide (1.47 g, 10 mmol). After stirring at room temperature for 15 hr under anhydrous conditions, the reaction mixture was evaporated to dryness. The residue was triturated with MeOH (50 mL) and filtered. The filtrate was adsorbed onto silica gel (20 g). The excess solvent was evaporated. Co-evaporation with toluene (3 x 50 mL) from the solid mass gave dry residue, which was loaded onto a silica gel column (4 x 40 cm) packed in CH_2Cl_2. The column was eluted with CH_2Cl_2:MeOH (9:1, v/v). The homogeneous fractions were pooled and evaporated to yield 2.50 g (60%) of the title compound as amorphous foam.

Chromatography: Absorbent - silica gel

Solvent - CH_2Cl_2:MeOH, 9:1, v/v

Infrared (KBr): Major bands - 1700, 1760, 3200 - 3500 $cm.^{-1}$

^{1}H NMR (Me_2SO-d_6): δ 1.32 and 1.48 (2s, 6, $2CH_3$), 6.38 (d, 1, J = <0.5 Hz, $C_{1'}H$), 7.70-7.88 (m, 6, $CONH_2$).

		C	H	N
Analysis:	Calcd for $C_{19}H_{19}N_5O_6$:	55.20	4.63	16.94
	Found:	54.97	4.74	16.77

1-(5-Amino-5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (9b). Method A. A mixture of 8 (1.03 g, 2.5 mmol) and n-butylamine (2 mL) in MeOH (10 mL) was heated under reflux for 12 hr, and then evaporated to dryness. The residue was purified on a silica gel column (2.5 x 25 cm) using $CHCl_3$:MeOH (9:1, v/v) as the eluent, and crystallized from MeOH to yield 0.35 (50%) of 1-(2,3-O-isopropylidene-5-amino-5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (6c); mp >70°C (dec.).

Chromatography:	Absorbent - silica gel
	Solvent - EtOAc:EtOH:H_2O, 2:1:1, v/v
Infrared (KBr):	Major bands - 700, 880, 1030, 1080, 1100, 1160, 1190, 1220, 1285, 1390, 1470, 1600, 1690, 2940, 2980, 3120 - 3380 $cm.^{-1}$
^{1}H NMR (Me_2SO-d_6):	δ 1.32 and 1.49 (2s, 6, $2CH_3$), 6.22 (d, 1, J = 2.0 Hz, $C_{1'}H$), 7.66 and 7.88 (2br s, 2, $CONH_2$), 8.85 (s, 1, C_5H).

		C	H	N
Analysis:	Calcd for $C_{11}H_{17}N_5O_4 \cdot 1/2H_2O$:	45.20	6.20	23.96
	Found:	45.47	6.24	23.68

Deisopropylidenation of 6c with 80% acetic acid as described for 9a gave 5′-amino-5′-deoxyribavirin (9b) as hygroscopic solid in 80% yield; mp >130°C (dec.).

Chromatography:	Absorbent - silica gel
	Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase
Infrared (KBr):	Major bands - 1670, 3100 - 3400 $cm.^{-1}$
^{1}H NMR (Me_2SO-d_6):	δ 3.16 (br s, 2, $C_{5'}$-NH_2, exchanged with D_2O), 5.84 (d, 1, J = 3.7 Hz, $C_{1'}H$), 7.63 and 7.84 (2 br s, 2, $CONH_2$), 8.89 (s, 1, C_5H).

		C	H	N
Analysis:	Calcd for $C_8H_{13}N_5O_4 \cdot 1/2H_2O$+EtOH:	39.27	6.22	25.44
	Found:	39.35	5.98	25.44

Method B. A mixture of compound 9a (2.69 g, 10 mmol) and 10% Pd/C (2.0 g) in EtOH:H_2O (1:1, v/v, 200 mL) was hydrogenated for 3 hr at room temperature at 37 psi. The catalyst was removed by filtration through a Celite pad, and the filtrate was concentrated to a small volume. To the concentrate was added ether and the solid that separated was collected by filtration. The solid was washed with a small amount of cold 50% aqueous EtOH (2 x 10 mL), followed by ether and crystallized from aqueous ethanol as hygroscopic solid, 1.70 g (70%); mp >130°C (dec.). This compound was identical in all respects to 9b prepared by Method A.

1-(2,3-O-Isopropylidene-5-deoxy-5-acetylthio-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (4a). Triphenylphosphine (5.25 g, 20 mmol) and diisopropyl azodicarboxylate (4.16 g, 20 mmol) were dissolved in anhydrous cold (0-5°C) THF (50 mL). To the solution was added a mixture of 2′,3′-O-isopropylidene ribavirin[28] (5, 2.84 g, 10 mmol) and thiolacetic acid (1.43 mL, 20 mmol) in THF (25 mL), dropwise, with stirring. The reaction mixture was stirred at ice bath temperature for 1 hr and then at ambient temperature for an additional 1 hr. A clear yellow solution was obtained, which was evaporated to dryness. The residue was triturated with MeOH (50 mL) and filtered. The filtrate was adsorbed onto silica gel (20 g). The excess solvent was evaporated. Co-evaporation with toluene (3 x 50 mL) from the solid mass gave dry residue, which was loaded onto a silica gel column (4 x 40 cm) packed in CH_2Cl_2. The column was eluted with CH_2Cl_2:MeOH (9:1, v/v). The homogeneous fractions were pooled and evaporated to yield 3.13 g (91.5%) of the title compound; mp 105-110°C.

Chromatography:	Absorbent - silica gel
	Solvent - EtOAc:H_2O:nPrOH, 4:2:1, upper phase
Infrared (KBr):	Major bands - 1590, 1680, 2980 and 3440 $cm.^{-1}$

^{1}H NMR (Me_2SO-d_6): δ 1.32 and 1.48 (2s, 6, $2CH_3$), 2.32 (s, 3, $SCOCH_3$), 6.32 (d, 1, J = 0.5 Hz, $C_{1'}H$), 7.68 and 7.88 (2 br s, 2, $CONH_2$), 8.84 (s, 1, C_5H).

		C	H	N	S
Analysis:	Calcd for $C_{13}H_{18}N_4O_5S.1/2H_2O$:	44.44	5.45	15.94	9.12
	Found:	44.48	5.21	15.71	8.96

1-(2,3-O-Isopropylidene-5-deoxy-5-thio-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (4b). A solution of 4a (17.12 g, 50 mmol) in absolute MeOH (500 mL) was adjusted to pH 9 with 1N NaOMe in MeOH and the resulting mixture was stirred at room temperature for 3 days with the exclusion of moisture. The reaction mixture was neutralized with Amberlite-IRC 120(H^+) ion-exchange resin. The resin was removed by filtration and the filtrate evaporated to dryness. The residual foam was purified on a silica gel column (4 x 40 cm) using $CHCl_3$:MeOH (6:1 v/v) as the eluent. The desired homogeneous fractions were pooled, solvent evaporated and the residue was crystallized from MeOH to yield 7.20 g (48%) of 4b; mp >85°C (foams).

Chromatography: Absorbent - silica gel
Solvent - $CHCl_3$:MeOH, 6:1, v/v

Infrared (KBr): Major bands - 690, 750, 865, 1020, 1070, 1090, 1150, 1175, 1205, 1270, 1370, 1440, 1480, 1590, 1670, 1685, 2920 and 3450 $cm.^{-1}$

^{1}H NMR (Me_2SO-d_6): δ 1.32 and 1.48 (2s, 6, $2CH_3$), 6.32 (d, 1, J = 0.5 Hz, $C_{1'}H$), 7.68 and 7.82 (2 br s, 2, $CONH_2$), 8.82 (s, 1, C_5H).

		C	H	N	S
Analysis:	Calcd for $C_{11}H_{16}N_4O_4S$:	43.99	5.37	18.66	10.69
	Found:	43.72	5.10	18.46	10.50

1-(5-Deoxy-5-thio-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (7). A suspension of compound 4b (3.0 g, 10 mmol) in 80% acetic acid (100 mL) was heated on a steam bath for 2 hr. A clear solution thus obtained was evaporated to dryness and the residue was purified on a silica gel column (2.5 x 40 cm) using $CHCl_3$:MeOH (6:1, v/v) as the eluent. Crystallization of the homogeneous product from MeOH gave 2.10 g (80%) of the title compound; mp >100°C (foams).

Chromatography: Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 6:1, v/v

Infrared (KBr): Major bands - 700, 760, 830, 1020, 1080, 1110, 1180, 1280, 1440, 1460, 1480, 1590, 1670, 2920 and 3420 $cm.^{-1}$

^{1}H NMR (Me_2SO-d_6): δ 5.90 (d, 1, J = 3.5 Hz, C_1,H), 7.66 and 7.90 (2 br s, 2, $CONH_2$), 8.88 (s, 1, C_5H).

		C	H	N	S
Analysis:	Calcd for $C_8H_{12}N_4O_4S$:	36.92	4.65	21.53	12.32
	Found:	36.93	4.64	21.31	12.06

2-(2,3-O-Isopropylidene-5-O-p-tolylsulfonyl-β-D-ribofuranosyl)thiazole-4-carboxamide (11a). In a similar manner as for 6a, treatment of 2′,3′-O-isopropylidenetiazofurin[20] (10, 6.0 g, 20 mmol) with p-toluenesulfonyl chloride (4.20 g, 22 mmol) in anhydrous pyridine, and purification of the reaction product on a silica gel column (4 x 50 cm) using $CHCl_3$:MeOH (6:1, v/v) gave 6.30 g (69.3%) of 11a as homogeneous foam.

Chromatography: Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 8:2, v/v

Infrared (KBr): Major bands - 1175, 1360, 1670 and 3440 $cm.^{-1}$

Ultraviolet:	λ_{max} (pH 1 and 7) 224 nm (ε 21,000), 266 sh (9,000);
	λ_{max} (pH 11) 222 nm (ε 21,300), 266 sh (9,500).
^{1}H NMR (Me_2SO-d_6):	δ 1.36 and 1.58 (2s, 6, $2CH_3$), 2.44 (s, 1, CH_3), 5.22 (d, 1, J = 3.5 Hz, $C_{1'}H$), 6.40, 7.30-7.70 (m, 6, $CONH_2$ and aromatic protons), 8.12 (s, 1, C_5H).

Analysis:		C	H	N	S
	Calcd for $C_{19}H_{22}N_2O_7S_2$:	50.21	4.88	6.16	14.10
	Found:	50.36	5.07	6.31	13.89

2-(2,3-O-Isopropylidene-5-azido-5-deoxy-β-D-ribofuranosyl)thiazole-4-carboxamide (11b). A solution of 11a (5.91 g, 13 mmol) and lithium azide (1.90 g, 39 mmol) in dry DMF (120 mL) was stirred at 85-90°C for 20 hr and evaporated to dryness at 50°C. The residue was co-evaporated several times with EtOH and then triturated with cold water (150 mL). The gummy residue was extracted with EtOAc, dried (Na_2SO_4) and evaporated to dryness. The residue was purified on a silica gel column (2.5 x 40 cm) using $CHCl_3$:MeOH (6:1, v/v) as the eluent to give 3.50 g (82.7%) of 11b as homogeneous foam.

Chromatography:	Absorbent - silica gel
	Solvent - $CHCl_3$:MeOH, 8:2, v/v
Infrared (KBr):	Major bands - 1685, 2110 and 3080 - 3480 $cm.^{-1}$
Ultraviolet:	λ_{max} (pH 1) 238 nm (ε 6,800);
	λ_{max} (pH 7 and 11) 238 nm (ε 6,900).
^{1}H NMR (Me_2SO-d_6):	δ 1.32 and 1.52 (2s, 6, $2CH_3$), 5.28 (d, 1, J = 3.5 Hz, $C_{1'}H$), 7.58 and 7.76 (2 br s, 2, $CONH_2$), 8.30 (s, 1, C_5H).

		C	H	N	S
Analysis:	Calcd for $C_{12}H_{15}N_5O_4S$:	44.30	4.65	21.53	9.85
	Found:	44.60	4.73	21.37	9.63

2-(5-Azido-5-deoxy-β-D-ribofuranosyl)thiazole-4-carboxamide (12). In a similar manner as for 7, deisopropylidenation of 11b (3.57 g, 11 mmol) with 80% acetic acid (100 mL) gave 2.50 g (81.5%) of 12; mp 119-120°C.

Chromatography: Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 9:1, v/v

Infrared (KBr): Major bands - 790, 930, 1040, 1055, 1085, 1110, 1130, 1200, 1285, 1340, 1390, 1430, 1490, 1520, 1610, 1660, 2100, 2920 - 3420 $cm.^{-1}$

Ultraviolet: λ_{max} (pH 1 and 7) 237 nm (ε 7,700); λ_{max} (pH 11) 237 nm (ε 7,100).

^1H NMR (Me_2SO-d_6): δ 5.02 (d, 1, J = 3.5 Hz, $C_{1'}H$), 7.56 and 7.68 (2 br s, 2, $CONH_2$), 8.25 (s, 1, C_5H).

		C	H	N	S
Analysis:	Calcd for $C_9H_{11}N_5O_4S$:	37.89	3.89	24.55	11.24
	Found:	37.84	3.92	24.35	11.17

2-(5-Amino-5-deoxy-β-D-ribofuranosyl)thiazole-4-carboxamide (15). A mixture of compound 12 (2.56 g, 9 mmol) and 10% Pd/C (2.0 g) in EtOH:H_2O (1:1, v/v, 200 mL) was hydrogenated for 6 hr at room temperature at 35 psi. The catalyst was removed by filtration through a Celite pad, and the filtrate was evaporated to dryness. The residue was crystallized from MeOH to yield 1.50 g (66%) of 15 as colorless needles; mp 189-190°C.

Chromatography:	Absorbent - silica gel
	Solvent - $CHCl_3$:MeOH, 8:2, v/v
Infrared (KBr):	Major bands - 1650, 1670, 3100 - 3500 $cm.^{-1}$
Ultraviolet:	λ_{max} (pH 1) 238 nm (ε 6,630);
	λ_{max} (pH 7) 238 nm (ε 6,900);
	λ_{max} (pH 11) 237 nm (ε 6,400).
^{1}H NMR (Me_2SO-d_6):	δ 3.28 (br s, 1, $C_{5'}$-NH_2, exchanged with D_2O), 4.96 (d, 1, J = 5.5 Hz, $C_{1'}H$), 7.60 and 7.70 (2 br s, 2, $CONH_2$), 8.24 (s, 1, C_5H).

		C	H	N	S
Analysis:	Calcd for $C_9H_{13}N_3O_4S$:	41.69	5.05	16.21	12.37
	Found:	41.92	5.33	15.97	12.48

2-(2,3-O-Isopropylidene-5-deoxy-5-acetylthio-β-D-ribofuranosyl)thiazole-4-carboxamide (13a). In a similar manner as for 4a, treatment of 2-(2,3-O-isopropylidene-β-D-ribofuranosyl)thiazole-4-carboxamide[20] (10, 3.0 g, 10 mmol) with triphenylphosphine (5.25 g, 20 mmol), diisopropyl azodicarboxylate (4.16 g, 20 mmol) and thiolacetic acid (1.43 mL, 20 mmol) in anhydrous THF (50 mL) gave 3.0 g (84%) of 13a as colorless needles; mp 159-161°C (dec.).

Chromatography:	Absorbent - silica gel
	Solvent - $CHCl_3$:MeOH, 9:1, v/v
Infrared (KBr):	Major bands - 620, 750, 860, 970, 1045, 1060, 1090, 1125, 1150, 1200, 1220, 1260, 1355, 1470, 1585, 1660, 2920, 2980 and 3460 $cm.^{-1}$
Ultraviolet:	λ_{max} (pH 1) 229 nm (ε 14,500);
	λ_{max} (pH 7) 228 nm (ε 14,700);
	λ_{max} (pH 11) 228 nm (ε 15,400).

^{1}H NMR (Me_2SO-d_6): δ 1.30 and 1.48 (2s, 6, $2CH_3$), 2.36 (s, 3, $SCOCH_3$), 5.26 (d, 1, J = 3.5 Hz, $C_{1'}H$), 7.58 and 7.66 (2 br s, 2, $CONH_2$), 8.26 (s, 1, C_5H).

		C	H	N	S
Analysis:	Calcd for $C_{14}H_{18}N_2O_5S_2$:	46.91	5.06	7.82	17.89
	Found:	47.17	5.07	7.88	18.02

2-(2,3-O-Isopropylidene-5-deoxy-5-thio-β-D-ribofuranosyl)thiazole-4-carboxamide (13b). In a similar manner as for 4b, treatment of 13a (1.07 g, 3 mmol) with 1N NaOMe in MeOH gave 0.50 g (53%) of 13b as amorphous solid.

Chromatography: Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 8:2, v/v

Infrared (KBr): Major bands - 1670, 3340 - 3450 $cm.^{-1}$

Ultraviolet: λ_{max} (pH 1) 236 nm (ε 3,600);

λ_{max} (pH 7) 236 nm (ε 3,800);

λ_{max} (pH 11) 236 nm (ε 4,700).

^{1}H NMR (Me_2SO-d_6): δ 1.33 and 1.52 (2s, 6, $2CH_3$), 5.30 (d, 1, J = 3.5 Hz, $C_{1'}H$), 7.60 and 7.80 (2 br s, 2, $CONH_2$), 8.28 (s, 1, C_5H).

		C	H	N	S
Analysis:	Calcd for $C_{12}H_{16}N_2O_4S$:	45.55	5.10	8.85	20.27
	Found:	45.55	4.86	8.77	20.16

2-(5-Deoxy-5-thio-β-D-ribofuranosyl)thiazole-4-carboxamide (14). In a similar manner as for 7, treatment of 13b (0.94 g, 3 mmol) with 80% aqueous acetic acid (50 mL) at 100°C for 2 hr gave 0.70 g (84%) of 14 as needles (from aqueous EtOH); mp 236-238°C.

Chromatography:	Absorbent - silica gel
	Solvent - $CHCl_3$:MeOH, 8:2, v/v
Infrared (KBr):	Major bands - 1660, 3200 - 3400 $cm.^{-1}$
Ultraviolet:	λ_{max} (pH 1) 238 nm (ε 6,600);
	λ_{max} (pH 7) 238 nm (ε 6,900);
	λmax (pH 11) 238 nm (ε 7,200).
^{1}H NMR (Me_2SO-d_6):	δ 5.50 (d, 1, J = 5.5 Hz, $C_{1'}H$), 7.56 and 7.68 (2 br s, 2, $CONH_2$), 8.21 (s, 1, C_5H).

		C	H	N	S
Analysis:	Calcd for $C_9H_{12}N_2O_4S_2$:	39.12	4.38	10.14	23.20
	Found:	39.34	4.10	10.10	22.92

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxylic acid (17). To a solution of ribavirin (1.22 g, 5 mmol) in water (20 mL) was added 6N NaOH (3 mL) and the mixture was stirred at room temperature for 24 hr. Water was evaporated and the residue was triturated with EtOH (3 x 10 mL). The residual solid was dissolved in water (10 mL) and the solution neutralized with Dowex-50 (H^+) resin. The resin was removed by filtration, the filtrate evaporated to dryness and the residue was crystallized from water to yield 1.10 g (90%) of the title compound; mp 187°C.

Chromatography:	Absorbent - silica gel
	Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase
Infrared (KBr):	Major bands - 675, 735, 810, 1010, 1040, 1080, 1200, 1225, 1270, 1430, 1450, 1470, 1600, 1710, 2900 and 3400 $cm.^{-1}$
^{1}H NMR (Me_2SO-d_6):	δ 5.80 (d, 1, J = 3.5 Hz, $C_{1'}H$), 8.82 (s, 1, C_5H) and other sugar protons.

		C	H	N
Analysis:	Calcd for $C_8H_{11}N_3O_6.H_2O$:	36.50	4.97	15.96
	Found:	36.32	4.75	15.70

1-(5-O-Nicotinoyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (16). To a stirred solution of dry ribavirin (3.66 g, 15 mmol) in pyridine:*N*,*N*-dimethylformamide (1:1, v/v, 150 mL), cooled to 0°C in an ice bath was added, dropwise, freshly distilled nicotinoyl chloride (2.14 g, 15.15 mmol). The clear reaction mixture was stirred at 0°C for 15 hr at the end of which time water (15 mL) was added, and the solvents were evaporated at 50°C. The residue was dissolved in MeOH (50 mL) and adsorbed onto silica gel (10 g). The excess solvent was evaporated under reduced pressure. Co-evaporation with toluene (3 x 50 mL) from the solid mass gave dry residue, which was loaded onto a silica gel column (4 x 40 cm) packed in $CHCl_3$. The column was eluted with $CHCl_3$:MeOH (9:1, v/v). The appropriate homogeneous fractions were combined and the solvents evaporated. The residue was crystallized from MeOH to yield 2.30 g (44%) of the title compound; mp 178°C.

Chromatography:	Absorbent - silica gel
	Solvent - $CHCl_3$:MeOH, 6:3, v/v
Infrared (KBr):	Major bands - 700, 750, 910, 1030, 1060, 1120, 1195, 1290, 1380, 1430, 1475, 1500, 1600, 1700, 2960 - 3500 $cm.^{-1}$
Ultraviolet:	λ_{max} (pH 1) 258 nm (ε 8,000);
	λ_{max} (pH 7) 260 nm (ε 5,200);
	λ_{max} (pH 11) 259 nm (ε 5,200).
^{1}H NMR (Me_2SO-d_6):	δ 5.95 (d, 1, J = 2.5 Hz, $C_{1'}H$), 7.50-9.05 (m, 7, $CONH_2$, C_5H and pyridyl protons).

		C	H	N
Analysis:	Calcd for $C_{14}H_{15}N_5O_6$:	48.14	4.32	20.04
	Found:	48.09	4.60	19.84

2-β-D-Ribofuranosylthiazole-4-carboxylic acid (18). In a similar manner as for 17, saponification of tiazofurin (2.60 g, 10 mmol) with 6N NaOH (6 mL) at room temperature gave 18, yield 2.40 g (92%); mp 72°C (dec.).

Chromatography: Absorbent - silica gel
Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr): Major bands - 685, 745, 1040, 1100, 1220, 1330, 1445, 1485, 1700, 2920 and 3410 $cm.^{-1}$

Ultraviolet: λ_{max} (pH 1) 248 nm (ε 6,800);
λ_{max} (pH 7) 246 nm (ε 5,200);
λ_{max} (pH 11) 246 nm (ε 5,000).

		C	H	N	S
Analysis:	Calcd for $C_9H_{11}NO_6S$:	41.38	4.24	5.36	12.25
	Found:	41.61	4.12	5.62	12.46

1-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-1,2,4-triazol-3(2H)-one (20). A mixture of 1,2,4-triazol-3(2H)-one (19, 0.65 g, 7.7 mmol) and 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose (5.80 g, 11.5 mmol) in dry nitromethane (150 mL) was brought to reflux temperature whereupon freshly distilled boron trifluoride etherate (1.46 mL, 11.6 mmol) was added through the condenser by syringe. After 40 min an additional charge of the sugar (1.50 g, 2.9 mmol) and the catalyst (0.5 mL, 3.9 mmol) was added to the refluxing reaction mixture. After 90 min of total reaction time, the solution was cooled and evaporated to dryness. The residue was dissolved in ethyl acetate (200 mL) and washed with saturated aqueous $NaHCO_3$ solution (2 x 100 mL), followed by water (2 x 100 mL). After drying over anhydrous Na_2SO_4, the solvent was evaporated to dryness. The residue was crystallized from methanol to yield 2.52 g (62%), mp 255°C.

Chromatography:	Absorbent - silica gel			
	Solvent - $CHCl_3$:acetone, 8:2, v/v			
Infrared (KBr):	Major bands - 710, 935, 1025, 1070, 1110, 1180, 1265, 1310, 1450, 1490, 1600, 1720, 2840, 3060, 3160, 3440 $cm.^{-1}$			
Ultraviolet:	λ_{max} (MeOH) 228 nm (ε 24,600), 278 (1,300).			
		C	H	N
Analysis:	Calcd for $C_{28}H_{23}N_3O_8$:	63.51	4.37	7.93
	Found:	63.78	4.36	7.86

1-β-D-Ribofuranosyl-1,2,4-triazol-3(2H)-one (21). To a solution of 20 (5.29 g, 10 mmol) in MeOH (100 mL) was added NaOMe till the pH of the solution was between 9-10, and the mixture was stirred at room temperature for 18 hr with the exclusion of moisture. After neutralization with Dowex-50 (H^+) resin, the reaction mixture was evaporated to dryness. The residue was dissolved in water (100 mL), the aqueous solution was extracted with ether (3 x 50 mL), and then evaporated to dryness. The residue was triturated with anhydrous ether, the solid that separated was collected and crystallized from aqueous ethanol to yield 1.41 g (65%) of 21, mp 155°C.

Chromatography:	Absorbent - silica gel
	Solvent - $CHCl_3$:MeOH, 6:1, v/v
Infrared (KBr):	Major bands - 730, 860, 910, 950, 1000, 1050, 1110, 1130, 1220, 1300, 1320, 1340, 1420, 1565, 1710, 1725, 2940, 3080, 3150 and 3420 $cm.^{-1}$

^{1}H NMR (Me_2SO-d_6): δ 5.40 (d, 1, J = 3.5 Hz, $C_{1'}H$), 7.84 (s, 1, C_5H).

		C	H	N
Analysis:	Calcd for $C_7H_{11}N_3O_5$:	38.70	5.10	19.34
	Found:	38.60	5.15	19.19

1-(2-Deoxy-3,5-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)pyrrole-2-carbonitrile (24). To a solution of pyrrole-2-carbonitrile[62] (22 , 0.92 g, 10 mmol) in dry CH_3CN (35 mL) was added NaH (60% in oil, 0.48 g, 12 mmol) and the mixture was stirred at room temperature under a nitrogen atmosphere for 30 min. 1-Chloro-2-deoxy-3,5-di-O-p-toluoyl-α-D-erythro-pentofuranose[63] (23, 3.88 g, 10 mmol) was added portionwise with stirring. The reaction mixture was stirred at room temperature for 0.5 h and at 50°C for 0.5 h, cooled and filtered to remove a small amount of insoluble material. Evaporation of the filtrate gave an oily residue, which was purified by flash chromatography using hexane:acetone (7:3, v/v) as the eluent to yield 3.0 g (67%) of 24 as needles, mp 125-128°C.

Chromatography: Absorbent - silica gel
Solvent - hexane:acetone, 9:1, v/v

Infrared (KBr): Major bands - 690, 735, 755, 855, 900, 960, 980, 1020, 1065, 1100, 1180, 1210, 1270, 1315, 1375, 1415, 1450, 1615, 1710, 1725, 2220, 2900 and 2960 cm^{-1}

Ultraviolet: λ_{max} (pH 1) 266 nm (ε 6,400);
λ_{max} (pH 7) 264 nm (ε 15,000);
λ_{max} (pH 11) 260 nm (ε 16,000).

^{1}H NMR ($CDCl_3$): δ 2.41 and 2.43 (2s, 6, $2CH_3$), 6.24 (t, 1, J = 6 Hz, $C_{1'}H$), 6.85 (d, 1), 7.11 (d, 1), 7.25 (m, 4, Ph), 7.93 (m, 4, Ph).

		C	H	N
Analysis:	Calcd for $C_{26}H_{24}N_2O_5$:	70.26	5.44	6.29
	Found:	70.18	5.43	6.20

1-(2-Deoxy-β-D-erythro-pentofuranosyl)pyrrole-2-carbonitrile (26). A suspension of 24 (11.0 g, 24.8 mmol) in MeOH/NH_3 (saturated at 0°C, 250 mL) was stirred at room temperature in a pressure bottle for 12 hr. The reaction mixture was cooled to 0°C, and evaporated to dryness. The residue was purified by flash chromatography using $CHCl_3$:acetone (8:2, v/v) as the eluent. The homogeneous product was crystallized from ether to yield 5.0 g (97%) of 26, mp 76-79°C.

Chromatography:	Absorbent - silica gel
	Solvent - $CHCl_3$:acetone, 8:2, v/v
Infrared (KBr):	Major bands - 750, 880, 925, 980, 1000, 1050, 1070, 1150, 1190, 1230, 1300, 1350, 1410, 1430, 1450, 2220, 2910, 2960, 3140 and 3380 $cm.^{-1}$
Ultraviolet:	λ_{max} (pH 1) 250 nm (ε 15,700);
	λ_{max} (pH 7) 250 nm (ε 14,500);
	λ_{max} (pH 11) 255 nm (ε 15,600).

		C	H	N
Analysis:	Calcd for $C_{10}H_{12}N_2O_3$:	57.69	5.81	13.45
	Found:	57.54	5.70	13.34

1-(2-Deoxy-β-D-erythro-pentofuranosyl)pyrrole-2-carboxamide (25a). A solution of 26 (3.2 g) in water (50 mL) was mixed with NH_4OH (120 mL) and 30% H_2O_2 (5 mL). The mixture was stirred at room temperature for 12 hr and evaporated to dryness. The residue was dissolved in MeOH (50 mL), adsorbed onto silica gel (10 g) and placed on top of a silica gel column

(6 x 40 cm). The column was eluted with $CHCl_3$:MeOH (6:2, v/v). The fractions containing the desired product were pooled and evaporated to dryness to amorphous foam to yield 2.80 g (81%).

Chromatography:	Absorbent - silica gel
	Solvent - $CHCl_3$:MeOH, 6:2, v/v
Infrared (KBr):	Major bands - 775, 1020, 1265, 1380, 1420, 1590, 1645, 2830, 2940 and 3350 $cm.^{-1}$
Ultraviolet:	λ_{max} (pH 1) 260 nm (ε 22,100);
	λ_{max} (pH 7) 260 nm (ε 24,200);
	λ_{max} (pH 11) 260 nm (ε 23,700).

		C	H	N
Analysis:	Calcd for $C_{10}H_{14}N_2O_4$:	53.09	6.24	12.38
	Found:	53.14	6.61	12.46

1-(2-Deoxy-β-D-erythro-pentofuranosyl)pyrrole-2-thiocarboxamide (25b). A solution of 26 (1.50 g) in anhydrous pyridine (150 mL) was saturated with H_2S at room temperature. After stirring the reaction mixture in a sealed vessel at room temperature for 12 hr, it was evaporated to dryness. The residue was purified by flash chromatography using $CHCl_3$:acetone (6:4, v/v) as the eluent to give 1.50 g (86%) of 25b, mp 126-128°C.

Chromatography:	Absorbent - silica gel
	Solvent - $CHCl_3$:acetone, 6:4 v/v
Infrared (KBr):	Major bands - 755, 850, 875, 930, 970, 1030, 1065, 1080, 1225, 1260, 1285, 1310, 1350, 1390, 1410, 1460, 1530, 1620, 1635, 2910, 3200, 3300 and 3380 $cm.^{-1}$
Ultraviolet:	λ_{max} (pH 1, 7 and 11) 279 nm (ε 10,000), 314 (16,000).

		C	H	N	S
Analysis:	Calcd for $C_{10}H_{14}N_2O_3S$:	49.59	5.83	11.56	13.21
	Found:	49.59	5.88	11.37	13.45

1-(2-Deoxy-β-D-erythro-pentofuranosyl)pyrrole-2-amidoxime (25c). A solution of 26 (2.20 g) and free NH_2OH (2.70 g) in absolute EtOH (200 mL) was heated under reflux for 3 hr and allowed to stir at room temperature overnight. Evaporation of the reaction mixture and purification of the residue by flash chromatograhy using $CHCl_3$:MeOH (8:2, v/v) as the eluent gave 2.20 g (86%) of 25c as foam.

Chromatography: Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 9:1, v/v

Infrared (KBr): Major bands - 690, 715, 930, 1040, 1080, 1270, 1310, 1360, 1415, 1590, 1630, 2910 and 3340 $cm.^{-1}$

Ultraviolet: λ_{max} (pH 1) 220 nm (ε 6,400), 269 (10,400); λ_{max} (pH 7 and 11) 242 nm (ε 7,300).

		C	H	N
Analysis:	Calcd for $C_{10}H_{15}N_3O_4$:	49.79	6.27	17.41
	Found:	49.50	6.25	17.28

1-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)pyrrole-2-carbonitrile (36). In a similar manner as for 24, glycosylation of the sodium salt of 22 (from 0.92 g, 10 mmol of 22 and 0.48 g, 12 mmol of 60% NaH) with 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide[65] (35) gave the title compound as foam, yield 2.4 g (45%).

Chromatography: Absorbent - silica gel

Solvent - hexane:acetone, 6:2, v/v

Infrared (KBr):	Major bands - 725, 770, 895, 985, 1030, 1060, 1090, 1170, 1220, 1260, 1310, 1360, 1430, 1440, 1485, 1575, 1590, 1715, 2210 and 3060 $cm.^{-1}$
Ultraviolet:	λ_{max} (pH 1) 260 nm (ε 4,700); λ_{max} (pH 7) 222 nm (ε 10,200); λ_{max} (pH 11) 270 nm (ε 3,000).

		C	H	N
Analysis:	Calcd for $C_{31}H_{24}N_2O_7$:	69.39	4.51	5.22
	Found:	69.54	4.52	4.99

Pyrrole-2,4-dicarbonitrile (27). This compound was prepared according to the procedure of Loader and Anderson,[62] mp 178-180°C.

Chromatography:	Absorbent - silica gel Solvent - $CHCl_3$:acetone, 8:2, v/v
Infrared (KBr):	Major bands - 720, 830, 850, 970, 1130, 1240, 1420, 1550, 1680, 2240, 3140, 3160, 3330 and 3420 $cm.^{-1}$
Ultraviolet:	λ_{max} (pH 1) 247 nm (ε 6,900); λ_{max} (pH 7) 248 nm (ε 6,800); λ_{max} (pH 11) 256 nm (ε 10,100).

		C	H	N
Analysis:	Calcd for $C_6H_3N_3$:	61.55	2.58	35.87
	Found:	61.32	2.46	36.10

1-(2-Deoxy-3,5-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)pyrrole-2,4-dicarbonitrile (28). In a similar manner as for 24, condensation of the sodium salt of 27 (from 1.17 g, 10 mmol of 27 and 60% NaH, 0.48 g, 12 mmol) with 23 (3.88 g, 10 mmol) in CH_3CN (50 mL) gave the title compound; yield

3.20 g (68%), mp 119-121°C.

Chromatography:	Absorbent - silica gel
	Solvent - hexane:acetone, 8:2, v/v
Infrared (KBr):	Major bands - 750, 835, 1020, 1100, 1180, 1270, 1385, 1445, 1485, 1610, 1710, 2240, 2930, 2960, 3140 and 3440 $cm.^{-1}$
Ultraviolet:	λ_{max} (pH 1) 260 nm (ε 15,000);
	λ_{max} (pH 7) 257 nm (ε 18,300);
	λ_{max} (pH 11) 264 nm (ε 9,900).

		C	H	N
Analysis:	Calcd for $C_{27}H_{23}N_3O_5$:	69.07	4.94	8.95
	Found:	69.22	4.95	9.04

1-(2-Deoxy-β-D-erythro-pentofuranosyl)pyrrole-2,4-dicarbonitrile (30). In a similar manner as for 26, treatment of 28 (13.5 g) with $MeOH/NH_3$ (300 mL) at room temperature gave 5.0 g (74%) of the title compound as homogeneous foam.

Chromatography:	Absorbent - silica gel
	Solvent - $CHCl_3$:acetone, 8:2, v/v
Infrared (KBr):	Major bands - 2220 and 3300 - 3350 $cm.^{-1}$
Ultraviolet:	λ_{max} (EtOH) 230 nm (ε 7,300), 247 (7,000).
^1H NMR (Me_2SO-d_6):	δ 6.12 (t, 1, J = 6.0 Hz, $C_{1'}H$), 7.56 (s, 1, C_5H), 8.30 (s, 1, C_3H).

		C	H	N
Analysis:	Calcd for $C_{11}H_{12}N_3O_3$:	56.41	5.16	17.93
	Found:	56.14	5.01	17.85

1-(2-Deoxy-β-D-erythro-pentofuranosyl)pyrrole-2,4-dicarboxamide (29a). In a similar manner as for 25a, hydration of 30 (4.2 g) with NH_4OH (150 mL) in the presence of 30% H_2O_2 (10 mL) gave the title compound, yield 4.0 g (83%), as foam.

Chromatography:	Absorbent - silica gel
	Solvent - $CHCl_3$:MeOH, 8:2, v/v
Infrared (KBr):	Major bands - 680, 740, 1020, 1045, 1080, 1270, 1370, 1400, 1480, 1585, 1640, 2840, 2910, 3020 and 3400 $cm.^{-1}$
Ultraviolet:	λ_{max} (pH 1, 7, and 11) 250 nm (ε 7,500).
^{1}H NMR (Me_2SO-d_6):	δ 6.83 (t, 1, J = 6.0 Hz, $C_{1'}H$), 6.91 and 7.12 (2s, 2, $CONH_2$), 7.11 (s, 1, C_5H), 7.44 and 7.67 (2s, 2, $CONH_2$).

		C	H	N
Analysis:	Calcd for $C_{11}H_{15}N_3O_5 \cdot 1/2H_2O$:	47.48	5.79	15.09
	Found:	47.24	5.96	14.94

1-(2-Deoxy-β-D-erythro-pentofuranosyl)pyrrole-2,4-bisthiocarboxamide (29b). In a similar manner as for 25b, treatment of 30 (2.0 g) with H_2S in pyridine (150 mL) containing Et_3N (5 mL) gave the title compound after silica gel flash chromatography using $CHCl_3$:MeOH, yield 1.60 g (62%), mp 184-186°C.

Chromatography:	Absorbent - silica gel
	Solvent - $CHCl_3$:MeOH, 9:1, v/v
Infrared (KBr):	Major bands - 685, 840, 875, 895, 945, 985, 1050, 1065, 1080, 1095, 1125, 1180, 1220, 1290, 1340, 1440, 1485, 1540, 1615, 1630, 2940, 3170 and 3380 $cm.^{-1}$
Ultraviolet:	λ_{max} (pH 1, 7 and 11) 248 nm (ε 9,500), 267 (10,000), 315 (15,100).

^{1}H NMR (Me_2SO-d_6): δ 6.99 (s, 1, C_5H), 7.01 (t, 1, J = 6.5 Hz, $C_{1'}H$), 7.95 (s, 1, C_3H), 8.99 and 9.22 (2s, 2, $CSNH_2$), 9.31 and 9.56 (2s, 2, $CSNH_2$).

Analysis:		C	H	N	S
	Calcd for $C_{11}H_{15}N_3O_3S_2$:	43.85	5.02	13.94	21.24
	Found:	43.99	5.10	13.80	21.04

1-(2-Deoxy-β-D-erythro-pentofuranosyl)pyrrole-2,4-bisamidoxime (29c). In a similar manner as for 25c, treatment of 30 (2.0 g) with free NH_2OH (3.0 g) in EtOH (200 mL) at reflux temperature gave 29c after silica gel flash chromatography using $CHCl_3$:MeOH (1:1, v/v), yield 1.8 g (70%), isolated as amorphous foam.

Chromatography: Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 9:1, v/v

Infrared (KBr): Major bands - 700, 760, 825, 950, 1060, 1100, 1210, 1285, 1340, 1380, 1440, 1480, 1590, 1630, 1720, 2920, 3030 and 3350 $cm.^{-1}$

Ultraviolet: λ_{max} (pH 1) 216 nm (ε 18,600), 262 (9,200); λ_{max} (pH 7 and 11) 245 nm (ε 13,000).

^{1}H NMR (Me_2SO-d_6): δ 5.48 (s, 2, NH_2), 5.65 (s, 2, NH_2), 6.54 (s, 1, C_5H), 6.62 (t, 1, J = 6.0 Hz, $C_{1'}H$), 7.45 (s, 1, C_3H), 9.06 (s, 1, NOH), 9.58 (s, 1, NOH).

Analysis:		C	H	N
	Calcd for $C_{11}H_{17}N_5O_5$:	44.15	5.73	23.39
	Found:	44.01	5.88	23.25

2-Amino-5-bromopyrrole-3,4-dicarbonitrile (31a). Starting from tetracyanoethylene (25.6 g, 200 mmol) and anhydrous hydrogen bromide gas, and following the procedure of Middleton et al.,[64] 13.8 g (33%) of the title compound was obtained, mp >250°C (dec.).

Chromatography:	Absorbent - silica gel
	Solvent - $CHCl_3$:acetone, 1:1, v/v
Infrared (KBr):	Major bands - 710, 860, 1075, 1120, 1255, 1360, 1415, 1480, 1540, 1600, 1630, 2210 and 2980 - 3440 $cm.^{-1}$
Ultraviolet:	λ_{max} (pH 1) 280 nm (ε 7,000);
	λ_{max} (pH 7) 254 sh, nm (ε 6,400), 282 (8,600);
	λ_{max} (pH 11) 248 nm (ε 6,400), 287 (9,300).

		C	H	N	Br
Analysis:	Calcd for $C_6H_3BrN_4$:	34.15	1.43	26.55	37.87
	Found:	34.02	1.58	26.73	37.66

2-Amino-5-mercaptopyrrole-3,4-dicarbonitrile (31b). 2,5-Diaminothiophene-3,4-dicarbonitrile (3.28 g) was rearranged with 10% NaOH solution according to the procedure of Middleton et al.[64] to obtain 2.0 g of the title compound as yellowish-brown solid, mp >220°C (dec.).

Chromatography:	Absorbent - silica gel
	Solvent - EtOAc:H_2O:*n*-PrOH, 4:2:1, upper phase
Infrared (KBr):	Major bands - 700, 760, 1460, 1500, 1530, 1600, 1640, 2210, 2920, 3020, 3240, 3340 and 3440 $cm.^{-1}$
Ultraviolet:	λ_{max} (pH 1) 287 nm (ε 11,200);
	λ_{max} (pH 7) 307 nm (ε 17,800);
	λ_{max} (pH 11) 295 nm (ε 7,100).

		C	H	N
Analysis:	Calcd for $C_6H_4N_4S$:	43.90	2.46	34.14
	Found:	44.01	2.27	33.81

5-Bromo-2-ethoxymethylenamino-1-(2-deoxy-3,5-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)pyrrole-3,4-dicarbonitrile (34a). A mixture of 31a (7.0 g, 33 mmol) and diethoxymethylacetate (8.10 g, 50 mmol) in dry CH_3CN (250 mL) was heated under reflux for 3 hr, cooled and evaporated to dryness. The residue was dissolved in a mixture of dry CH_3CN (50 mL) and toluene (50 mL), and evaporated to dryness. This process was repeated three times and the residual 2-ethoxymethylenamino-5-bromopyrrole-3,4-dicarbonitrile (32a, 8.0 g, 90%) was used as such for further reactions.

Treatment of the sodium salt of 32a (from 8.10 g, 30.3 mmol of the nitrile and 1.40 g, 35 mmol of 60% NaH in oil) with 23 (11.70 g, 30.5 mmol) in CH_3CN (400 mL), in the same manner as for 24, gave 14.0 g (74.5%) of 34a as foam.

Chromatography:	Absorbent - silica gel
	Solvent - hexane:acetone, 7:3, v/v
Infrared (KBr):	Major bands - 750, 840, 995, 1020, 1100, 1180, 1270, 1370, 1450, 1515, 1620, 1715, 2220, 2920, 2980 and 3420 $cm.^{-1}$
Ultraviolet:	λ_{max} (pH 1) 240 nm (ε 3,100);
	λ_{max} (pH 7) 237 nm (ε 5,100);
	λ_{max} (pH 11) 230 nm (ε 2,500).
^{1}H NMR ($CDCl_3$):	δ 1.42 (t, 3, CH_2CH_3), 2.41 (2s, 6, $2CH_3$), 4.48 (m, 2, CH_2CH_3), 6.39 (t, 1, J = 7.0 Hz, $C_{1'}H$), 7.24 - 7.90 (m, 8, 2 Ph), 8.27 (s, 1, CH).

		C	H	N	Br
Analysis:	Calcd for $C_{30}H_{27}BrN_4O_6$:	58.16	4.39	9.04	12.89
	Found:	58.40	4.46	8.95	13.11

4-Amino-6-bromo-7-(2-deoxy-β-D-erythro-pentofuranosyl)pyrrolo[2,3-d]-pyrimidine-5-carbonitrile (33a). A solution of 34a (0.80 g, 1.3 mmol) in

$MeOH/NH_3$ (50 mL) was stirred at room temperature in a pressure bottle for 2 days and then evaporated to dryness. The residue was purified by flash chromatography using $CHCl_3$:MeOH (8:2, v/v) as the eluent and crystallized from $CHCl_3$/MeOH mixture to yield 0.35 g (76.5%) of 33a, mp >300°C (dec.).

Chromatography: Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 8:2, v/v

Infrared (KBr): Major bands - 2215 and 3300 - 3400 $cm.^{-1}$

Ultraviolet: λ_{max} (pH 1) 233 nm (ϵ 15,400), 282 (15,400); λ_{max} (pH 7) 218 nm (ϵ 17,800), 286 (14,500); λ_{max} (pH 11) 286 nm (ϵ 16,100).

^{1}H NMR (Me_2SO-d_6): δ 6.42 (t, 1, J = 7.0 Hz, $C_{1'}H$), 7.04 (br s, 2, NH_2), 8.19 (s, 1, C_2H).

Analysis:		C	H	N	Br
	Calcd for $C_{12}H_{12}BrN_5O_3$:	40.69	3.42	19.76	22.56
	Found:	40.45	3.30	19.56	22.85

5-Ethylthio-2-ethoxymethylenamino-1-(2-deoxy-3,5-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)pyrrole-3,4-dicarbonitrile (34b). The title compound was prepared in a similar manner as described for 34a using 2-amino-5-ethylthiopyrrole-3,4-dicarbonitrile[64] (which was converted to 32b by boiling with diethoxymethylacetate) (6.5 g, 26 mmol), NaH (1.20 g, 30 mmol), 23 (11.7 g, 30 mmol) and dry CH_3CN (300 mL). The product was purified by flash chromatography using hexane:acetone (6:4, v/v), and crystallized from the same solvent to yield 12.0 g (76%), mp 129-131°C.

Chromatography: Absorbent - silica gel

Solvent - hexane:acetone, 6:2, v/v

Infrared (KBr): Major bands - 750, 840, 995, 1020, 1100, 1180, 1275, 1375, 1410, 1450, 1500, 1530, 1610, 1620, 1710, 2220, 2920, 2980 and 3420 $cm.^{-1}$

Ultraviolet: λ_{max} (MeOH) 236 nm (ε 33,300), 281 (11,200).

^{1}H NMR ($CDCl_3$): δ 1.27 and 1.43 (2t, 6, $2CH_2C\underline{H}_3$), 2.39 and 2.43 (2s, 6, $2C\underline{H}_3$), 4.47 (m, 4, $2C\underline{H}_2CH_3$), 6.62 (t, 1, J = 6.0 Hz, $C_1,\underline{H}$), 7.22 - 7.90 (m, 8, 2 Ph), 8.27 (s, 1, C$\underline{H}$).

		C	H	N	S
Analysis:	Calcd for $C_{32}H_{32}N_4O_6S$:	63.99	5.37	9.32	5.33
	Found:	64.23	5.25	9.33	5.60

4-Amino-6-ethylthio-1-(2-deoxy-β-D-erythro-pentofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carbonitrile (33b). In a similar manner as for 33a, the title compound was prepared by using 34b (6.5 g, 10.1 mmol) and $MeOH/NH_3$ (200 mL). The product was crystallized from MeOH:$CHCl_3$:ether mixture to yield 3.60 g (91.0%) of 33b, mp 177-179°C.

Chromatography: Absorbent - silica gel

Solvent - $CHCl_3$:acetone, 6:4, v/v

Infrared (KBr): Major bands - 690, 750, 790, 910, 930, 980, 1050, 1090, 1260, 1300, 1370, 1430, 1470, 1560, 1580, 1625, 2220, 2920, 3200, 3320 and 3420 $cm.^{-1}$

Ultraviolet: λ_{max} (pH 1) 235 nm (ε 11,300), 295 (11,800); λ_{max} (pH 7 and 11) 233 nm (ε 9,100), 295 (13,300).

^{1}H NMR (Me_2SO-d_6): δ 1.20 (t, 3, $CH_2C\underline{H}_3$), 3.0 (q, 2, $C\underline{H}_2CH_3$), 6.64 (t, 1, J = 6.6 Hz, $C_1,\underline{H}$), 7.07 (br s, 2, $N\underline{H}_2$), 8.22 (s, 1, $C_2\underline{H}$).

		C	H	N	S
Analysis:	Calcd for $C_{14}H_{17}N_5O_3S$:	50.15	5.11	20.84	9.54
	Found:	50.06	5.13	20.51	9.56

2-Amino-4-methylpyrrole-3-carbonitrile (38). Condensation of malononitrile (6.6 g, 100 mmol) with acetamidoacetone (11.5 g, 100 mmol) in the presence of NaOH, according to the procedure of Wamhoff and Wehling[66] gave the title compound, yield 8.72 g (72%), mp 121°C.

Chromatography: Absorbent - silica gel

Solvent - $CHCl_3$:acetone, 9:1, v/v

Infrared (KBr): Major bands - 720, 785, 1015, 1060, 1130, 1340, 1435, 1490, 1550, 1580, 1620, 2180, 2920 - 3380cm.$^{-1}$

Ultraviolet: λ_{max} (pH 1) 228 nm (ε 7,700);

λ_{max} (pH 7 and 11) 259 nm (ε 5,500).

		C	H	N
Analysis:	Calcd for $C_6H_7N_3$:	59.49	5.83	34.68
	Found:	59.21	5.75	34.45

3-Benzyloxy-1-β-D-ribofuranosylpyrazole-4-carbonitrile (44b). 3-Benzyloxy-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole-4-carbonitrile (44a, 4.47 g, 10 mmol) was combined with liquid NH_3 (50 mL) in a steel reaction vessel and allowed to stand at room temperature for 15 hr, after which NH_3 was evaporated. The dry residue was purified on a flash silica gel column (4 x 30 cm) using $CHCl_3$:MeOH (9:1, v/v) as the eluent, and crystallized from aqueous EtOH to yield 1.60 g (48.3) of 44b, mp 138°C.

Chromatography: Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 9:1, v/v

Infrared (KBr): Major bands - 680, 730, 860, 950, 1030, 1070, 1100, 1150, 1195, 1345, 1380, 1455, 1500, 1550, 2210, 2910, 3120 and 3480 $cm.^{-1}$

Ultraviolet: λ_{max} (pH 1 and 7) 240 nm (ε 19,000); λ_{max} (pH 11) 240 nm (ε 17,900).

^1H NMR (Me_2SO-d_6): δ 5.27 (s, 2, $CH_2C_6H_5$), 5.56 (d, 1, J = 4.0 Hz, $C_{1'}H$), 7.40 (m, 5, $CH_2C_6H_5$), 8.52 (s, 1, C_5H).

		C	H	N
Analysis:	Calcd for $C_{16}H_{17}N_3O_5$:	58.00	5.17	12.68
	Found:	57.76	5.13	12.44

3-Benzyloxy-1-β-D-ribofuranosylpyrazole-4-thiocarboxamide (43b). A slow stream of H_2S was bubbled through a solution of 44a (4.57 g, 10 mmol) in dry pyridine (125 mL) containing triethylamine (4 mL) for 3 hr. After stirring for 12 hr at room temperature, the mixture was evaporated to dryness. The residue was co-evaporated with EtOH (3 x 50 mL) and the residual syrup (43a, 3.20 g) was deacetylated without further purification. The above syrup (43a) was dissolved in $MeOH/NH_3$ (saturated at 0°C, 100 mL) and stirred at room temperature for 16 hr. $MeOH/NH_3$ was evaporated and the residue was purified on a silica gel column (2.5 x 50 cm) using $CHCl_3$:MeOH (6:1, v/v) as the eluent. The fractions containing the homogeneous product were pooled, evaporated and the residue was crystallized from EtOH to yield 0.80 g (21.9%), mp 132°C.

Chromatography: Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 6:1, v/v

Infrared (KBr): Major bands - 730, 760, 855, 910, 990, 1010, 1030, 1050, 1070, 1105, 1150, 1380, 1420, 1445

1500, 1565, 1610, 2880, 2960, 3320 and 3440 $cm.^{-1}$

Ultraviolet: λ_{max} (pH 1) 258 nm (ε 5,500), 303 (10,200); λ_{max} (pH 7) 258 nm (ε 4,000), 303 (7,300); λ_{max} (pH 11) 258 nm (ε 9,500), 303 (9,500).

^{1}H NMR (Me_2SO-d_6): δ 5.28 (s, 2, $CH_2C_6H_5$), 5.52 (d, 2, J = 3.5 Hz, $C_{1'}H$), 7.40 (m, 5, $CH_2C_6H_5$), 8.35 (s, 1, C_5H), 8.13 and 9.33 (2 br s, 2, $CSNH_2$).

Analysis:		C	H	N	S
	Calcd for $C_{16}H_9N_3O_5S$:	51.96	5.31	11.36	8.67
	Found:	51.78	5.53	11.26	8.39

3-Benzyloxy-1-β-D-ribofuranosylpyrazole-4-carboxylic acid (42a). To a solution of ethyl 3-benzyloxy-1-β-D-ribofuranosylpyrazole-4-carboxylate (1.89 g, 5 mmol) in H_2O (5 mL) was added 6N NaOH (3 mL) and the mixture was stirred at room temperature for 24 hr. Water was evaporated and the residue was triturated with EtOH (3 x 10 mL). The solid was dissolved in H_2O (10 mL) and the solution neutralized with Dowex-50 (H^+) resin. The resin was removed by filtration, the filtrate evaporated to dryness and the residue was crystallized from water to yield 1.50 g (85.6%) of the title compound, mp 138°C.

Chromatography: Absorbent - silica gel
Solvent - $CHCl_3$:MeOH, 6:1, v/v

Infrared (KBr): Major bands - 700, 720, 765, 845, 890, 970, 1070, 1100, 1165, 1250, 1350, 1440, 1500, 1560, 1680, 2920 and 3350 $cm.^{-1}$

Ultraviolet: λ_{max} (pH 1) 242 nm (ε 18,200), 303 (1,750); λ_{max} (pH 7) 238 nm (ε 11,900); λ_{max} (pH 11) 232 nm (ε 11,200).

^{1}H NMR (Me_2SO-d_6): δ 5.22 (s, 2, $CH_2C_6H_5$), 5.52 (d, 1, J = 4.0 Hz, $C_{1'}H$), 7.41 (m, 5, $CH_2C_6H_5$), 8.31 (s, 1, C_5H).

		C	H	N
Analysis:	Calcd for $C_{18}H_{18}N_2O_7$:	54.85	5.18	7.99
	Found:	54.70	5.20	7.90

3-Hydroxy-1-β-D-ribofuranosylpyrazole-4-carboxylic acid (42b). To a solution of 42a (1.75 g, 5 mmol) in 95% EtOH (100 mL) was added 10% Pd/C (0.10 g) and the mixture was shaken in a Parr hydrogenator for 5 hr at 45 psi of H_2. The mixture was filtered through a Celite pad and the filtrate evaporated to dryness. The residue was crystallized from aqueous EtOH to yield 1.06 g (81.5%) of 42b, mp >80°C (dec.).

Chromatography: Absorbent - silica gel
Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared(KBr): Major bands - 700, 780, 860, 900, 1050, 1080, 1130, 1175, 1290, 1445, 1485, 1530, 1570, 1690, 2920 and 3380 $cm.^{-1}$

Ultraviolet: λ_{max} (pH 1) 240 nm (ε 14,800);
λ_{max} (pH 7) 238 nm (ε 16,400);
λ_{max} (pH 11) 264 nm (ε 13,700).

^{1}H NMR (Me_2SO-d_6): δ 5.46 (d, 1, J = 4.5 Hz, $C_{1'}H$), 8.16 (s, 1, C_5H).

		C	H	N
Analysis:	Calcd for $C_9H_{12}N_2O_7 \cdot 1/2H_2O$:	40.15	4.86	10.40
	Found:	40.40	5.05	10.49

4-Amino-8-(β-D-ribofuranosylamino)pyrimido[5,4-d]pyrimidine (46). A solution of 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine-6-carbonitrile[77]

(45, 6.0 g, 14.8 mmol) in conc. NH_4OH (200 mL) was stirred at room temperature for 8 hr and then allowed to stand at 4°C for 16 hr. The mixture was evaporated to dryness. The crude product which had been absorbed onto silica gel (10 g) was loaded on a 3.5 x 35 cm silica gel column packed in EtOAc. The column was eluted with EtOAc:H_2O:n-PrOH (4:2:1, v/v, upper phase). The appropriate homogeneous fractions were pooled, and the solvent was evaporated to yield 3.3 g (80%) of white solid. Crystallization from H_2O gave thin fan-shaped plates, mp 214-216°C.

Chromatography: Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Ultraviolet: λ_{max} (pH 7) 292 nm (ε 16,000), 303 (14,100), 319 (12,400), 334 (8,500).

^1H NMR (Me_2SO-d_6): δ 5.88 (q, 1, $C_{1'}H$, which collapsed to a doublet at δ 5.88 after deuteration), 7.80 (br s, 2, NH_2), 8.40 and 8.50 (2s, C_2H and C_6H).

		C	H	N
Analysis:	Calcd for $C_{11}H_{14}N_6O_4$:	44.89	4.80	28.56
	Found:	45.01	4.96	28.45

6-Methylthio-9-β-D-ribofuranosylpurine (49). A solution of 9-β-D-ribofuranosyl-6-mercaptopurine (0.33 g) in 0.4 N NaOH (2.5 mL, 0.86 equiv) was shaken at room temperature for 10 min. while CH_3I (0.073 mL, 1 equiv) was added in portions. NaOH (0.4 mL, 0.4 N) was added and the solution again shaken with CH_3I (0.073 mL). The solution was kept at room temperature for 2 hr during which time white needles separated. After refrigeration overnight, the solid was collected, dried over NaOH and refluxed for several minutes with absolute EtOH (2 mL). Filtration of the chilled suspension gave white micro-needles (0.26 g, 74%), mp 164°C.

Chromatography: Absorbent - silica gel

Solvent - EtOAc:H_2O:*n*-PrOH, 4:2:1, upper phase

Infrared (KBr): Major bands - 660, 820, 865, 950, 990, 1030, 1060, 1100, 1210, 1330, 1400, 1440, 1480, 1570, 2880, 2950 and 3360 $cm.^{-1}$

Ultraviolet: λ_{max} (pH 1) 223 nm (ε 17,000), 293 (25,900);

λ_{max} (pH 7) 222 nm (ε 17,600), 289 (28,900);

λ_{max} (pH 11) 289 nm (ε 28,500).

Analysis:

	C	H	N	S
Calcd for $C_{11}H_{14}N_4O_4S$:	44.29	4.73	18.78	10.75
Found:	44.30	4.75	18.56	10.93

Neplanocin A (47). The culture filtrates of a fermentation broth produced by neplanocin A-producer CL-1018 was obtained from Warner-Lambert Pharmaceutical Research Division, Ann Arbor, Michigan. Isolation of the antibiotic neplanocin A was performed by the successive column chromatography on ion-exchange resin and charcoal as reported in the literature,[84] mp 205-207°C.

Chromatography: Absorbent - silica gel

Solvent - EtOAc:H_2O:*n*-PrOH, 4:2:1, upper phase.

Infrared (KBr): Major bands - 725, 865, 1060, 1120, 1290, 1335, 1410, 1570, 1600, 1650, 2850 and 3120 - 3380 $cm.^{-1}$

Ultraviolet: λ_{max} (pH 1) 259 nm (ε 14,500);

λ_{max} (pH 7) 260 nm (ε 15,300);

λ_{max} (pH 11) 260 nm (ε 15,500).

Analysis:

	C	H	N
Calcd for $C_{11}H_{13}N_5O_3$:	50.18	4.98	26.60
Found:	49.98	5.16	26.37

Purine-6-sulfonyl fluoride (51). To a cold (0°C) mixture of MeOH (500 mL), 49% HF (750 mL) and $KF.2H_2O$ (750 g) in a polyethylene beaker was added purine-6-thiol (125 g). While cooling and stirring a fast stream of Cl_2 gas was bubbled into the reaction mixture. The rate of Cl_2 introduction was adjusted so that a reaction temperature of -2 to +3°C was maintained. The time required for the reaction was about 5 hr. The reaction mixture was poured slowly with stirring onto 4 kg of crushed ice, stirred for 5 min and the pale yellow solid that separated was collected by filtration. The residue was washed thoroughly with ice-cold water, pressed dry, and air-dried. It was crystallized from boiling absolute EtOH using charcoal as white crystals, yield 108 g (65%), mp >300°C [Lit[80] mp >300°C].

Chromatography:	Absorbent - silica gel			
	Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase			
Infrared (KBr):	Major bands - 625, 800, 850, 910, 930, 970, 1140, 1170, 1210, 1300, 1370, 1400, 1550, 1590, 1865, and 2640 - 3100 $cm.^{-1}$			
Ultraviolet:	λ_{max} (pH 1) 282 nm (ε 4,650);			
	λ_{max} (pH 7) 280 nm (ε 4,050);			
	λ_{max} (pH 11) 278 nm (ε 5,050).			
		C	H	N
Analysis:	Calcd for $C_5H_3FN_4O_2S$:	29.70	1.48	27.72
	Found:	30.07	1.46	27.41

4-Amino-6-methyl-2-methylthiopyrrolo[2,3-d]pyrimidine (54). 4-Chloro-6-methyl-2-methylthiopyrrolo[2,3-d]pyrimidine[55] (55, 3.0 g, 14.0 mmol) was combined with $MeOH/NH_3$ (75 mL, saturated at 0°C) and the resulting mixture heated in a steel bomb at 135°C for 48 hr. The residue, after evaporation of the solvents, was crystallized from aqueous ethanol as needles to yield 1.64 g (60%) of 54, mp 207°C.

Chromatography:	Absorbent - silica gel
	Solvent - $CHCl_3$:acetone, 8:2, v/v.
Infrared (KBr):	Major bands - 780, 940, 970, 1150, 1240, 1275, 1300, 1350, 1380, 1460, 1535, 1595, 1610 and 2930 - 3400 $cm.^{-1}$
Ultraviolet:	λ_{max} (pH 1) 231 nm (ε 11,700), 285 (9,600); λ_{max} (pH 7 and 11) 233 nm (ε 15,300), 286 (10,200), 302 sh (8,700).
^{1}H NMR (Me_2SO-d_6):	δ 2.25 (s, 3, CH_3), 2.41 (s, 3, SCH_3), 6.08 (s, 1, C_5H), 6.77 (s, 2, NH_2), 11.20 (s, 1, N_7H).

Analysis:		C	H	N	S
	Calcd for $C_8H_{10}N_4S$:	49.46	5.19	28.84	16.50
	Found:	49.38	5.24	28.96	16.32

Methyl 2-chloro-5-cyanomethyl-3-methylimidazole-4-carboxylate (57). To a solution of methyl 2-chloro-5-cyanomethylimidazole-4-carboxylate[81] (56, 3.3 g, 16.5 mmol) in water (50 mL) containing NaOH (0.75 g) was added dimethylsulfate (1.88 mL, 20 mmol) and the mixture was stirred at ambient temperature for 4 hr. The mixture was evaporated to dryness and the residue was purified on a silica gel column (4 x 25 cm) using chloroform: methanol (6:1, v/v) as the solvent. The homogeneous product was crystallized from aqueous methanol to yield 2.2 g (62%) of 57, mp 84°C.

Chromatography:	Absorbent - silica gel
	Solvent - $CHCl_3$:MeOH, 6:1, v/v
Infrared (KBr):	Major bands - 770, 800, 850, 935, 1050, 1120, 1145, 1195, 1260, 1310, 1325, 1370, 1420, 1440, 1465, 1550, 1690, 2260 and 2960 $cm.^{-1}$

Ultraviolet:	λ_{max} (pH 1) 248 nm (ε 8,300);				
	λ_{max} (pH 7) 248 nm (ε 9,500);				
	λ_{max} (pH 11) 247 nm (ε 9,400).				
		C	H	N	Cl
Analysis:	Calcd for $C_8H_8ClN_3O_2$:	44.97	3.77	19.67	16.59
	Found:	45.00	3.74	19.71	16.60

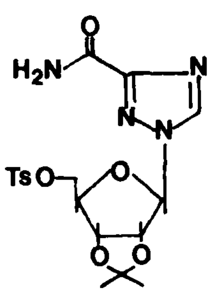
2,4-Dinitroimidazole[83] (59). 86% HNO_3 (30 mL) was cooled to 0°C and to this was added Ac_2O (10 mL) with stirring. After stirring for 10 min azomycin[82] (2.2 g, 20 mmol) was added in small portions. After the addition of azomycin, the reaction mixture was allowed to stir at 100°C for 2 hr and at 150°C for 30 min. After cooling the reaction mixture was poured over ice (150 g), the aqueous solution was extracted with EtOAc (2 x 75 mL), and washed with saturated brine solution (2 x 50 mL). The dried (Na_2SO_4) organic phase was evaporated to dryness and the residue was crystallized from hot methanol to yield 1.30 g (42%) of the title compound, mp 264-266°C.

Chromatography:	Absorbent - silica gel			
	Solvent - $CHCl_3$:MeOH, 8:2, v/v			
Infrared (KBr):	Major bands - 760, 805, 820, 840, 900, 1015, 1105, 1160, 1220, 1280, 1345, 1360, 1405, 1430, 1490, 1520, 1550 and 2800 - 3160 $cm.^{-1}$			
Ultraviolet:	λ_{max} (pH 1) 307 nm (ε 5,400);			
	λ_{max} (pH 7) 354 nm (ε 10,100);			
	λ_{max} (pH 11) 356 nm (ε 8,800).			
		C	H	N
Analysis:	Calcd for $C_3H_2N_4O_4$:	22.80	1.27	35.45
	Found:	23.05	1.33	35.25

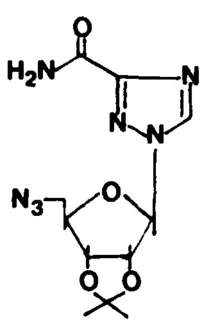
IV. LIST OF COMPOUNDS SUBMITTED TO WALTER REED ARMY INSTITUTE OF RESEARCH (WRAIR) FROM SEPTEMBER 1, 1984 THROUGH AUGUST 31, 1985

During the progress report period, September 1, 1984 through August 31, 1985, the following forty-one (41) compounds were prepared and submitted to Chemical Handling and Data Analysis Branch, Division of Experimental Therapeutics, Department of Medicinal Chemistry, Walter Reed Army Institute of Research, Washington, D.C. for antiviral evaluation, each in pure form. In addition to these compounds, 2.0 g of 4-amino-8-(β-D-ribofuranosylamino)-pyrimido[5,4-d]pyrimidine (BJ-76187) has been submitted for indepth antiviral screening. The chemical structure of each of these compounds is shown below:

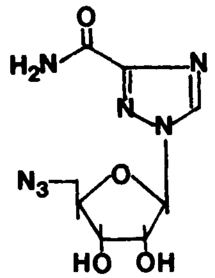
No.	Compound	Qty.	Notebook No.	WRAIR No.	Ref.
1.	1-(2,3-O-Isopropylidene-5-O-p-tolylsulfonyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide	2.00 g	SY-3	BL-00334	p23



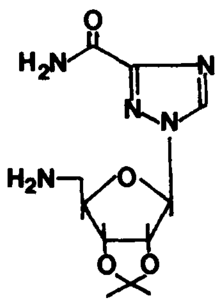
No.	Compound	Qty.	Notebook No.	WRAIR No.	Ref.
2.	1-(2,3-O-Isopropylidene-5-azido-5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide	2.00 g	SY-6	BL-00325	p24



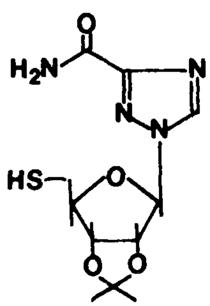
3. 1-(5-Deoxy-5-azido-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide 1.50 g SY-28 BL-07333 p25



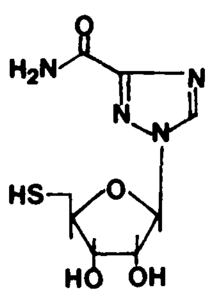
4. 1-(2,3-O-Isopropylidene-5-deoxy-5-amino-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide 1.50 g SY-7 BL-04181 p26



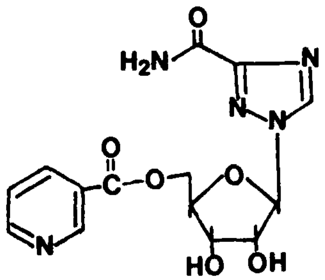
5. 1-(2,3-O-Isopropylidene-5-deoxy-5-thio-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide 1.60 g SY-8 BL-04190 p29



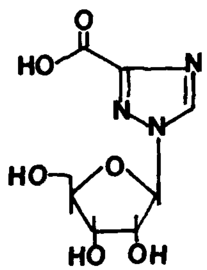
6. 1-(5-Deoxy-5-thio-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide 1.60 g SY-15 BL-04207 p30



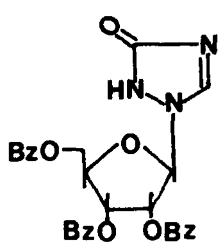
7.	1-(5-O-Nicotinoyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide	1.80 g	SR-162	BK-98937	p36



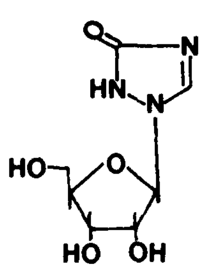
8.	1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxylic acid	1.90 g	SR-179	BL-00281	p85



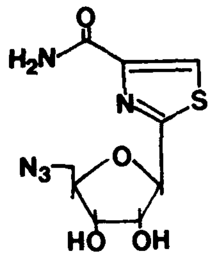
9.	1-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-1,2,4-triazol-3(2H)-one	2.00 g	SR-164	BL-00307	p37



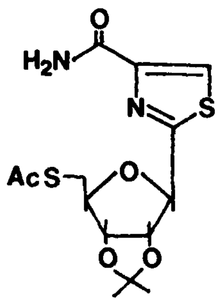
10.	1-β-D-Ribofuranosyl-1,2,4-triazol-3(2H)-one	2.00 g	SR-165	BL-00290	p38



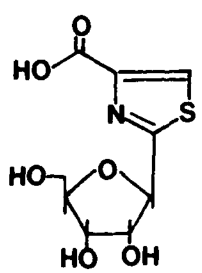
11. 2-(5-Deoxy-5-azido-β-D-ribofuranosyl)thiazole-4-carboxamide | 1.50 g | SY-41 | BL-07342 | p32



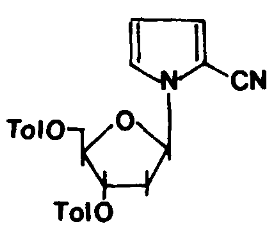
12. 2-(5-Deoxy-5-acetylthio-2,3-di-O-isopropylidene-β-D-ribofuranosyl)-thiazole-4-carboxamide | 2.00 g | SY-22 | BL-07351 | p33



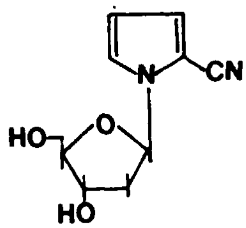
13. 2-β-*D*-*Ribofuranosylthiazole-4-*carboxylic acid | 1.80 g | SR-178 | BL-00272 | p37



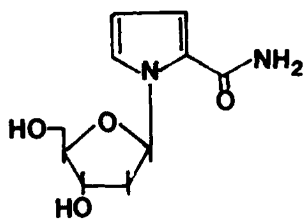
14. 1-(2-Deoxy-3,5-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)-pyrrole-2-carbonitrile | 1.80 g | SV-29 | BK-98900 | p39



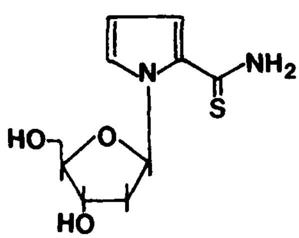
15. 1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)pyrrole-2-carbonitrile 1.80 g SV-43 BL-00361 p40



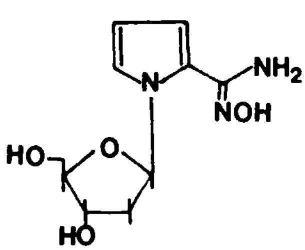
16. 1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)pyrrole-2-carboxamide 2.00 g SV-45 BL-04145 p40



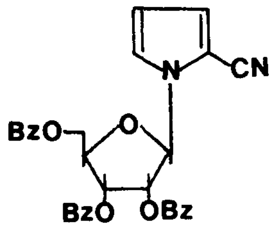
17. 1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)pyrrole-2-thiocarboxamide 1.10 g SV-95 BL-04154 p41



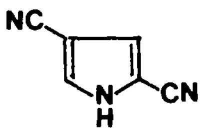
18. 1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)pyrrole-2-amidoxime 1.50 g SV-94 BL-07360 p42



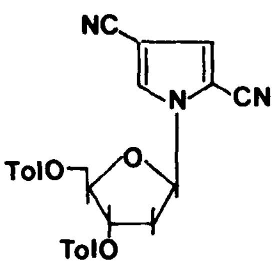
19. 1-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)pyrrole-2-carbonitrile — 2.00 g — SV-33 — BL-00370 — p42



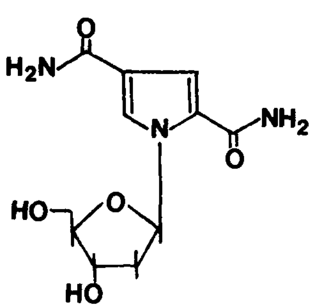
20. Pyrrole-2,4-dicarbonitrile — 1.80 g — SV-20 — BK-98884 — 62



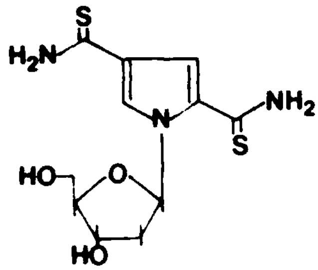
21. 1-(2-Deoxy-3,5-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)pyrrole-2,4-dicarbonitrile — 1.80 g — SV-30 — BK-98893 — p43



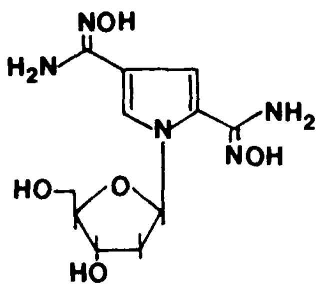
22. 1-(2-Deoxy-β-D-erythro-pentofuranosyl)pyrrole-2,4-dicarboxamide — 1.70 g — SV-53 — BL-00352 — p44



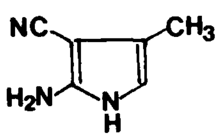
23. 1-(2-Deoxy-β-D-erythro-pentofuranosyl)pyrrole-2,4-bisthiocarboxamide — 1.30 g — SV-135 — BL-07388 — p45



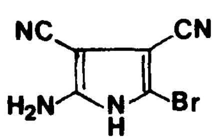
24. 1-(2-Deoxy-β-D-erythro-pentofuranosyl)pyrrole-2,4-bisamidoxime — 1.30 g — SV-138 — BL-07397 — p46



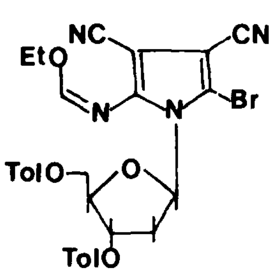
25. 2-Amino-4-methylpyrrole-3-carbonitrile — 1.50 g — SV-14 — BK-98866 — 66



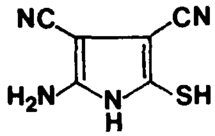
26. 2-Amino-5-bromopyrrole-3,4-dicarbonitrile — 1.70 g — SV-35 — BK-98875 — 64



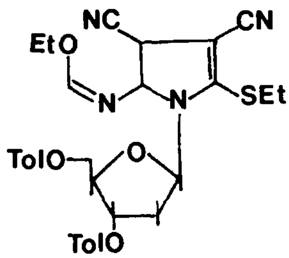
27. 5-Bromo-2-ethoxymethylenamino-1-(2-deoxy-3,5-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)pyrrole-3,4-dicarbonitrile — 1.30 g — SV-64 — BL-07379 — p48



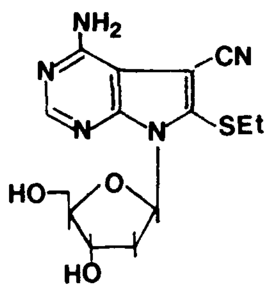
28.	2-Amino-5-mercaptopyrrole-3,4-dicarbonitrile	1.80 g	SV-62	BL-00343	64



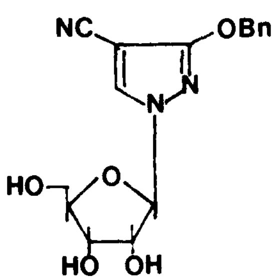
29.	5-Ethylthio-2-ethoxymethylen-amino-1-(2-deoxy-3,5-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)pyrrole-3,4-dicarbonitrile	1.80 g	SV-97	BL-04163	p49



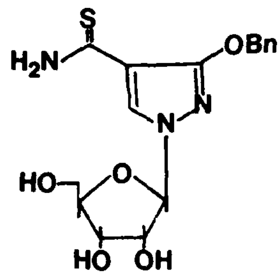
30.	4-Amino-6-ethylthio-1-(2-deoxy-β-D-erythro-pentofuranosyl)-pyrrole[2,3-d]pyrimidine-5-carbonitrile	1.20 g	SV-99	BL-04172	p50



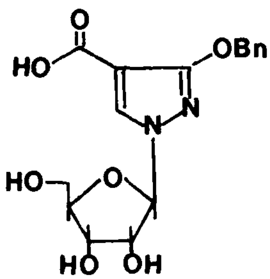
31.	3-Benzyloxy-1-β-D-ribofuranosyl-pyrazole-4-carbonitrile	1.00 g	SR-142	BK-96675	p51



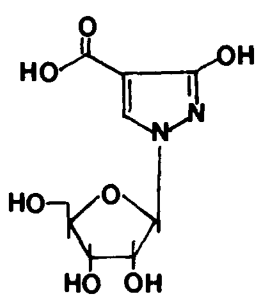
32. 3-Benzyloxy-1-β-D-ribofuranosyl-pyrazole-4-thiocarboxamide 1.10 g SR-132 BK-96666 p52



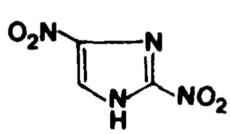
33. 3-Benzyloxy-1-β-D-ribofuranosyl-pyrazole-4-carboxylic acid 2.00 g SR-170 BL-04127 p53



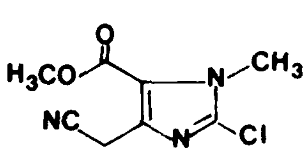
34. 3-Hydroxy-1-β-D-ribofuranosyl-pyrazole-4-carboxylic acid 1.10 g SR-187 BL-04136 p54



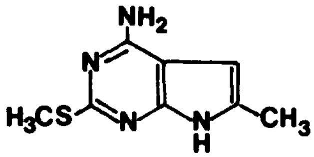
35. 2,4(5)-Dinitroimidazole 1.50 g SV-21 BK-98919 83



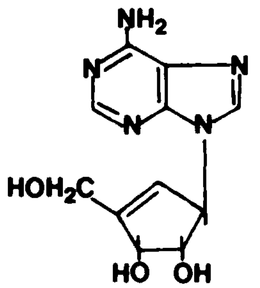
36. Methyl 2-chloro-5-cyanomethyl-3-methylimidazole-4-carboxylate 1.10 g SN-198 BK-96684 p58



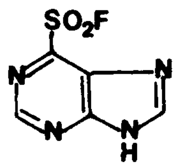
37. 4-Amino-6-methyl-2-methylthio-pyrrolo[2,3-d]pyrimidine 1.20 g RE-673 BK-96693 55



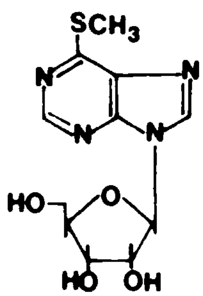
38. Neplanocin A 1.50 g RA-300 BL-04118 84



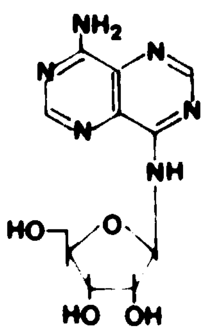
39. Purine-6-sulfonyl fluoride 2.00 g SY-2 BL-00263 80



40. 6-Methylthio-9-β-D-ribofurano-sylpurine 1.10 g RA-228c BK-98928 p55



41. 4-Amino-8-(β-D-ribofuranosyl-amino)pyrimido[5,4-d]pyrimidine 2.00 g RA-149 BJ 76187 p54



V. REFERENCES

1. S. S. Cohen, Science, 197, 431 (1977).

2. A. S. Galabov, Arzneim. Forsch., 26, 169 (1976).

3. G. D. Diana and F. Pancic, Angew. Chem. Intl. Ed. (English), 15, 410 (1976).

4. W. M. Shannon and F. M. Schabel, Jr., Pharmacol. Ther., 11, 263 (1980).

5. T. H. Maugh, Science, 192, 128 (1976).

6. W. H. Prusoff and D. C. Ward, Biochem. Pharmacol., 25, 1233 (1976).

7. R. K. Robins, Chem. Eng. News, 64, 28 (Jan. 27, 1986).

8. R. W. Sidwell, L. N. Simon, J. T. Witkowski and R. K. Robins, Progr. Chemother., Proc. 8th Intl. Congr. Chemother., 2, 889 (1974).

9. S. Harris and R. K. Robins, in "Ribavirin, A Broad Spectrum Antiviral Agent," pp 1-21, Eds. R. A. Smith and W. Kirkpatrick, Academic Press (1980).

10. R. W. Sidwell, G. R. Revankar and R. K. Robins, Viral Chemother., 2, 49 (1985).

11. Y.-C. Cheng, B. Goz, J. P. Neenan, D. C. Ward and W. H. Prusoff, J. Virol., 15, 1284 (1975).

12. Y.-C. Cheng, J. P. Neenan, B. Goz, D. C. Ward and W. H. Prusoff, Ann. N. Y. Acad. Sci., 255, 322 (1975).

13. W. H. Prusoff, D. C. Ward, T. S. Lin, M. S. Chen, G. T. Shaiu, C. Chai, E Lentz, R. Capizzi, J. Idriss, N. H. Ruddle, F. L. Black, H. L. Kumari, D. Albert, P. N. Bhatt, G. D. Hsiung, S. Strickland and Y.-C. Cheng, Ann. N. Y. Acad. Sci., 284, 335 (1977).

14. M. S. Chen, D. C. Ward and W. H. Prusoff, J. Biol. Chem., 251, 4833 (1976).

15. T-S. Lin, C. Chai and W. H. Prusoff, J. Med. Chem., 19, 915 (1976).

16. W. Jahn, Chem. Ber., 98, 1705 (1965).

17. W. H. Shannon and F. Schabel, Jr., Pharmacol. Ther., 11, 263 (1980).

18. P. Prusiner and M. Sundaralingam, Nature New Biol., 244, 116 (1973).

19. P. C. Srivastava, M. V. Pickering, L. B. Allen, D. G. Streeter, M. T. Campbell, J. T. Witkowski, R. W. Sidwell and R. K. Robins, J. Med. Chem., 20, 256 (1977).

20. M. Fuertes, M. T. Garcia-Lopez, G. Garcia-Munoz and M. Stud, J. Org. Chem., 41, 4074 (1976).

21. H. N. Jayaram, R. L. Dion, R. I. Glazer, G. D. Johns, R. K. Robins, P. C. Srivastava and D. A. Cooney, Biochem. Pharmacol., 31, 2371 (1982).

22. M. F. Earle and R. I. Glazer, Cancer Res., 43, 133 (1983).

23. G. Gebeyehu, V. E. Marquez, A. V. Cott, D. A. Cooney, J. A. Kelley, H. N. Jayaram, G. S. Ahluwalia, R. L. Dion, Y. A. Wilson and D. G. Johns, J. Med. Chem., 23, 99 (1985).

24. R. K. Robins, P. C. Srivastava, V. L. Narayanan, J. Plowman and K. D. Paull, J. Med. Chem., 25, 107 (1982).

25. P. C. Srivastava, G. R. Revankar and R. K. Robins, J. Med. Chem., 27, 266 (1984).

26. R. K. Robins and G. R. Revankar, Med. Res. Reviews 5, 273 (1985).

27. J. T. Witkowski and R. K. Robins, in "Chemistry and Biology of Nucleosides and Nucleotides," R. E. Harmon, R. K. Robins, L. B. Townsend, Eds., Academic Press, New York, p. 267 (1978).

28. M. Fuertes, J. T. Witkowski, D. G. Streeter and R. K. Robins, J. Med. Chem., 17, 642 (1974).

29. A. M. Michelson and A. R. Todd, J. Chem. Soc., 816 (1955).

30. J. P. Horwitz, A. J. Tomson, J. A. Urbanski and J. Chua, J. Org. Chem., 27, 3045 (1962).

31. M. G. Stout, M. J. Robins, R. K. Olsen and R. K. Robins, J. Med. Chem., 12, 658 (1969).

32. O. Mitsunobu, S. Takizawa and H. Morimato, J. Am. Chem. Soc., 98, 7858 (1976).

33. R. P. Volante, Tetrahedron Lett., 22, 3119 (1981).

34. D. C. Baker, T. H. Haskell and S. R. Putt, J. Med. Chem., 21, 1218 (1978).

35. J. M. Knoblich, J. M. Sugihara and T. Yamazaki, J. Org. Chem., 36, 3407 (1971).

36. A. H. Haines, Adv. Carbohydr. Chem. Biochem., 33, 11 (1976).

37. M. J. Robins, W. A. Bowles and R. K. Robins, J. Am. Chem. Soc., 86, 1251 (1964).

38. M. J. Robins and R. K. Robins, J. Am. Chem. Soc., 87, 4934 (1965).

39. J. T. Witkowski, M. Fuertes, P. D. Cook and R. K. Robins, J. Carbohydr. Nucleosides Nucleotides, 2, 1 (1975).

40. M. J. Robins and R. K. Robins, J. Org. Chem., 34, 2160 (1969).

41. For a review of the synthesis of 2′-deoxynucleosides, see: (a) L. Goodman, in "Basic Principles in Nucleic Acid Chemistry"; P. O. P. Ts'o, Ed., Academic Press, New York (1974), Vol. 1, pp 93-208. (b) J. G. Moffatt, in "Nucleoside Analogues: Chemistry, Biology and Medical Applications"; R. T. Walker, E. De Clercq and F. Eckstein, Eds., Plenum Press, New York (1979), pp 71-164.

42. A. M. Mian and T. A. Khwaja, J. Med. Chem., 26, 286 (1983).

43. L. F. Christensen, A. D. Broom, M. J. Robins and A. Bloch, J. Med. Chem., 15, 735 (1972).

44. G. E. Wright and L. W. Dudycz, J. Med. Chem., 27, 175 (1984).

45. M. J. Robins and J. S. Wilson, J. Am. Chem. Soc., 103, 932 (1981).

46. M. J. Robins, J. S. Wilson and F. Hansske, J. Am. Chem. Soc., 105, 4059 (1983).

47. R. A. Lessor and N. J. Leonard, J. Org. Chem., 46, 4300 (1981).

48. K. Pankiewicz, A. Matsuda and K. A. Watanabe, J. Org. Chem., 47, 485 (1982).

49. K. Fukukawa, T. Ueda and T. Hirano, Chem. Pharm. Bull., 31, 1842 (1983).

50. M. C. Huang, K. Hatfield, A. W. Roetker, J. A. Montgomery and R. L. Blakely, Biochem. Pharmacol., 30, 2663 (1981).

51. T. Maruyama, L. L. Wotring and L. B. Townsend, J. Med. Chem., 26, 25 (1983).

52. Z. Kazimierczuk, G. R. Revankar and R. K. Robins, Nucleic Acids Res., 12, 1179 (1984).

53. Z. Kazimierczuk, H. B. Cottam, G. R. Revankar and R. K. Robins, J. Am. Chem. Soc., 106, 6379 (1984).

54. G. R. Revankar, P. K. Gupta, A. D. Adams, N. K. Dalley, P. A. McKernan, P. D. Cook, P. G. Canonico and R. K. Robins, J. Med. Chem., 27, 1389 (1984).

55. H. B. Cottam, Z. Kazimierczuk, S. Geary, P. A. McKernan, G. R. Revankar and R. K. Robins, J. Med. Chem., 28, 1461 (1985).

56. P. K. Gupta, R. K. Robins and G. R. Revankar, Nucleic Acids Res., 13, 5341 (1985).

57. P. K. Gupta, N. K. Dalley, R. K. Robins and G. R. Revankar, J. Heterocycl. Chem., 23, 59 (1986).

58. M. Kawana and S. Emoto, Bull. Chem. Soc. Jpn., 41, 2552 (1968).

59. M. Kawana and S. Emoto, Bull. Chem. Soc. Jpn., 42, 3539 (1969).

60. M. Kawana, Chem. Lett., 1541 (1981).

61. For a review of "indoline-indole" method see: (a) M. N. Preobrazhenskaya, I. A. Korbukh, V. N. Tolkachev, Ja. V. Dobrynin and G. I. Vornovitskaya, INSERM, Nucleosides, Nucleotides Biol. Appl., 81, 85 (1978). (b) G. R. Revankar and R. K. Robins, in "Chemistry of Nucleosides and Nucleotides", L. B. Townsend, Ed., Plenum Press, New York, in press.

62. C. E. Loader and H. J. Anderson, Can. J. Chem., 59, 2673 (1981).

63. M. Hoffer, Chem. Ber., 93, 2777 (1960).

64. W. J. Middleton, V. A. Engelhardt and B. S. Fisher, J. Am. Chem. Soc., 80, 2822 (1958).

65. J. D. Stevens, R. K. Ness and H. G. Fletcher, Jr., J. Org. Chem., 33, 1806 (1968).

66. H. Wamhoff and B. Wehling, Synthesis, 51 (1976).

67. G. E. Gutowski, M. J. Sweeney, D. C. DeLong, R. L. Hamill, K. Gerzon and R. W. Dyke, Ann. N.Y. Acad. Sci., 255, 544 (1975).

68. J. Descamps and E. De Clercq, "Current Chemotherapy"; W. Siegenthaler, R. Luthy, Eds., American Society for Microbiology, Washington, D. C. (1978), p. 354.

69. E. De Clercq and P. F. Torrence, J. Carbohydr. Nucleosides Nucleotides, 6, 187 (1978).

70. W. M. Shannon, Ann. N.Y. Acad. Sci., 284, 472 (1977).

71. F. J. Streightoff, J. A. Nelson, J. C. Cline, K. Gerzon, M. Hoehn, R. H. Williams, M. Gorman and D. C. DeLong, 9th Intersci. Confer. Antimicrob. Agents Chemother., Abstr. 18 (1969).

72. D. C. DeLong, L. D. Baker, K. Gerzon, G. E. Gutowski, R. H. Williams and R. L. Hamill, 7th Int. Congr. Chemother., A-5/35 (1971).

73. E. J. Reist, V. J. Bartuska and L. Goodman, J. Org. Chem., 29, 3725 (1964).

74. K. D. Philips and J. P. Horwitz, J. Org. Chem., 40, 1856 (1975).

75. J. D. Westover, G. R. Revankar, R. K. Robins, R. D. Madsen, J. R. Ogden, J. A. North, R. W. Mancuso, R. J. Rousseau and E. L. Stephen, J. Med. Chem., 24, 941 (1981).

76. Antiviral screening data from U. S. Army Med. Res. Inst. of Infect. Dis., letter from J. W. Huggins to R. K. Robins, July 12, 1982.

77. H. M. Berman, R. J. Rousseau, R. W. Mancuso, G. P. Kreishman and R. K. Robins, Tetrahedron Lett., 3099 (1973).

78. M. Hayashi, S. Yaginuma, N. Muto and M. Tsujino, Nucleic Acids Res. Symp. Ser., 8, 565 (1980).

79. Commercially available from Aldrich Chemical Co., Milwaukee.

80. A. G. Beaman and R. K. Robins, J. Am. Chem. Soc., 83, 4038 (1961).

81. P. D. Cook, R. J. Rousseau, A. M. Mian, P. Dea, R. B. Meyer, Jr. and R. K. Robins, J. Am. Chem. Soc., 98, 1492 (1976).

82. S. Nakamura, Chem. Pharm. Bull., 3, 379 (1955).

83. G. C. Lancini, N. Maggi and P. Sensi, Farmaco (Pavia), Ed. Sci., 18, 390 (1963).

84. M. Hayashi, S. Yaginuma, N. Muto and M. Tsujino, Nucleic Acids Res., Sym. Ser., No. 8 (1980).

85. International Tables for X-Ray Crystallography, Vol. 4, J. A. Ibers and W. D. Hamilton; Eds. Kynoch Press, Birmingham, England, 1974, p. 99.

86. G. M. Sheldrick; "SHELXTL. An Integrated System for Solving, Refining and Displaying Crystal Structures from Diffraction Data"; 4th Revision, University of Gottingen, Federal Republic of Germany, 1983.

VI. STAFFING

Contract No. DAMD17-79-C-9046

During the report period the following personnel have been engaged in the work on the contract:

Name	Effort
Roland K. Robins, Ph.D. Principal Investigator	
Ganapathi R. Revankar, Ph.D. Co-Investigator	60% - Sept. 1, 1984 to Aug. 31, 1985
Kandasamy Ramasamy, Ph.D. Postdoctoral Research Fellow	100% - Sept. 1, 1984 to Aug. 31, 1985
Krishna G. Upadhya, Ph.D. Postdoctoral Research Fellow	100% - Sept. 1, 1984 to Aug. 31, 1985
Naeem B. Hanna, Ph.D. Postdoctoral Research Fellow	100% - Dec. 1, 1984 to Aug. 31, 1985
Jack Anderson, B.S. Graduate Research Assistant	50% - Jan. 1, 1985 to Aug. 31, 1985
Mark Smith, B.S. Graduate Research Assistant	50% - Feb. 1, 1985 to Aug. 31, 1985
A. David Adams, B.S. Technician	40% - Sept. 1, 1984 to Aug. 31, 1985
Miland Gadekar Lab Technician	50% - Sept. 1, 1984 to Aug. 31, 1985

Date: Sept. 15, 1986

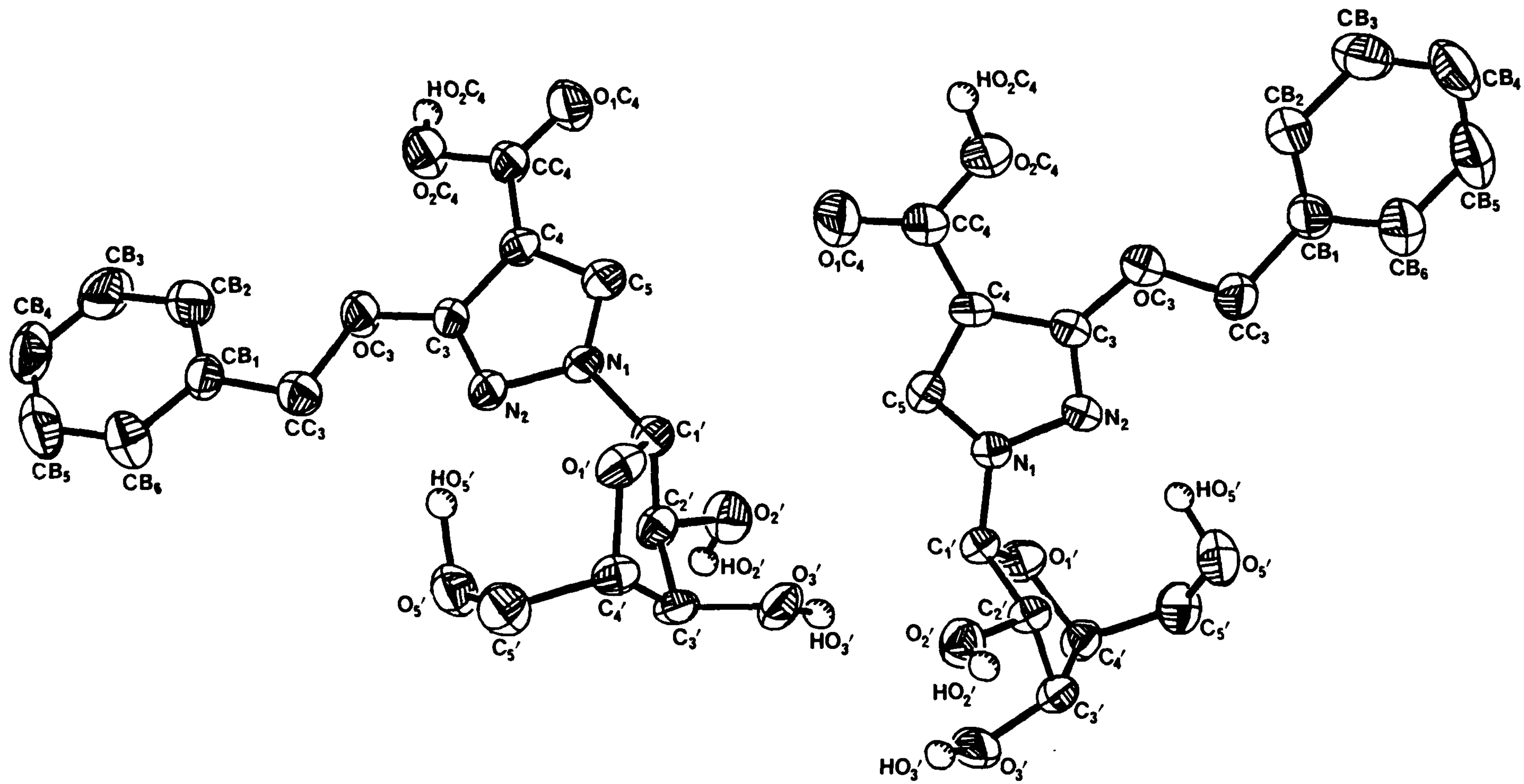
VII. APPENDIX

Single-Crystal X-ray Diffraction Analysis of Compound 42a. A suitable crystal of the compound was mounted on a Nicolet P3 autodiffractometer which utilized graphite monochromated Cu radiation (λ = 1.54178 Å). An attempt to calculate lattice parameters and an orientation matrix from 25 centered reflections failed suggesting that the crystal was twinned. It was possible to select fifteen reflections of one orientation suitable for lattice parameters and an orientation matrix was calculated from these values. Crystal and Structure data are shown in Table I. Single-crystal intensity data were obtained using a variable scan speed θ - 2θ procedure. Sixty-one data were rejected as the backgrounds were measured on peaks of the twin, however a large majority of data was acceptable. Three check reflections were measured every 97 reflections. There was no systematic change in these data indicating crystal and electronic stability. The data were merged to 2491 independent reflections, 96 of which were considered unobserved as $I < 2\sigma(I)$. The structure was solved using direct methods and refined using a blocked cascading least squares procedure. Density calculations as well as the structure determination indicated that there were two molecules in the asymmetric unit. All nonhydrogen atoms were refined anisotropically. Positions for hydrogen atoms bonded to carbon atoms were calculated based on stereochemical considerations. These atoms were allowed to ride on their neighboring carbon atoms during refinement. The thermal parameter of each of these hydrogen atoms was fixed at 1.2 times the initial equivalent isotropic thermal parameter of the neighboring atom. All hydrogens bonded to oxygen atoms were located in difference maps. The positional parameters of these atoms were not refined but the atoms were refined isotropically. An empirical extinction correction was made as several of the low angle reflections had larger F calculated than F observed. The resulting R values were R = 0.049 and R_w = 0.067 with weights based on counting statistics. The largest peaks in the final difference maps were 0.37 $e\text{Å}^{-3}$ and -0.32$e\text{Å}^{-3}$. Scattering factors were obtained from the "International Tables for X-ray Crystallography".[85] All computer calculations and the computer drawing were performed using SHELXTL.[86]

A computer drawing of the two crystallographically independent molecules of <u>61</u> with atom labels and conformation is shown in <u>Figure 1</u>. The bond lengths are listed in <u>Table II</u>. The positional and thermal parameters of the atoms are listed in <u>Table III</u>. There is good agreement between chemically similar bonds. It is quite evident that both the molecules are in the *β conformation*. The aglycon portions of both molecules are planar. The largest deviation of a base ring atom from the least-squares plane of the base in 0.002Å for N_2 in molecule A and 0.010Å for C_3 in molecule B. The carboxylic acid group deviates more from the plane of its base in molecule A than in molecule B as the dihedral angle between the planes of the base and carboxylic acid group is 15.2° in molecule A compared to 5.4° in molecule B.

The carbohydrate moiety of the two molecules are nearly identical. Both exist in the ${}^{3}_{2}T$ conformation with corresponding torsion angles of the two carbohydrate moieties differing by no more than 2.1°. In both glycon moieties there is an intramolecular hydrogen bond $O_{5'}$-$HO_{5'}$,N_2 (see <u>Figure 1</u> and <u>Table IV</u>). This hydrogen bond causes the respective molecules to exist in a rather compact form. The glycosidic torsion angles N_2-N_1-$C_{1'}$-$C_{2'}$ of the two molecules are very similar with a value of 60.1° in molecule A and 61.1° in molecule B.

There is a rather extensive hydrogen bonding network which links the two molecules (Table IV). All of the hydrogen atoms bonded to oxygen atoms of the two molecules, with the exception of HO_2, in molecule B, are involved in hydrogen bonds. Oxygen of O_1C_4 of molecule A interacts with two hydrogen atoms, while the corresponding oxygen of molecule B does not participate in any hydrogen bonding. This may account for the large dihedral angle between the plane of the carboxylic acid group and the aglycon in molecule A, as mentioned above.



Molecule A

Molecule B

Computer drawing of twinned crystal of 42a

(The hydrogen atoms bonded to carbon atoms are omitted for clarity)

Figure I

TABLE I

Crystal and Structure Data of Compd 42a

Formula	$C_{16}H_{18}N_2O_7$
Molecular Weight	350.32
F(000)	368
μ, cm^{-1}	9.18
Crystal size (mm)	0.3 x 0.3 x 0.1
Space group	P1
a, Å	4.857(2)
b, Å	10.071(9)
c, Å	16.889(6)
α, deg	97.48(4)
β, deg	95.56(3)
γ, deg	90.19(4)
V, $Å^3$	815(1)
Z	2
d, g cm^{-1}	1.43
radiation	Cu (1.54178 A)
sin θ/λ max	0.54
unique observed data	2395
unobserved data	96
R	0.049
R_w	0.067
largest peaks in Δ F map, e$Å^{-3}$	0.37, -0.32

TABLE II

Bond lengths (Å) with e.s.d.[a] values in parenthesis

	Molecule A	Molecule B
N_1-N_2	1.375(4)	1.378(5)
N_2-C_3	1.310(4)	1.315(5)
C_3-OC_3	1.336(5)	1.332(6)
OC_3-CC_3	1.426(6)	1.434(5)
CC_3-CB_1	1.501(6)	1.499(7)
C_3-C_4	1.427(6)	1.429(6)
C_4-CC_4	1.450(6)	1.456(6)
CC_4-O_1C_4	1.211(6)	1.218(6)
CC_4-O_2C_4	1.323(6)	1.289(7)
O_2C_4-HO_2C_4	0.98[a]	0.86
C_4-C_5	1.379(6)	1.386(6)
C_5-N_1	1.331(6)	1.326(5)
N_1-$C_{1'}$	1.458(5)	1.442(5)
$C_{1'}$-$C_{2'}$	1.537(6)	1.525(6)
$C_{2'}$-$O_{2'}$	1.404(5)	1.411(5)
$O_{2'}$-$HO_{2'}$	0.95	0.92
$C_{2'}$-$C_{3'}$	1.511(6)	1.529(6)
$C_{3'}$-$O_{3'}$	1.430(5)	1.425(6)
$O_{3'}$-$HO_{3'}$	0.94	0.97
$C_{3'}$-$C_{4'}$	1.497(6)	1.508(7)
$C_{4'}$-$C_{5'}$	1.516(6)	1.511(7)
$C_{5'}$-$O_{5'}$	1.411(6)	1.410(7)
$O_{5'}$-$HO_{5'}$	1.17	1.05
$C_{4'}$-$O_{1'}$	1.450(5)	1.499(5)
$O_{1'}$-$C_{1'}$	1.383(6)	1.408(5)
average C-C in benzene	1.379(13)	1.378(18)

[a] e.s.d. value on bond lengths involving H atoms is estimated at 0.03Å. The positioned parameters of these atoms were not refined.

TABLE III

Positional (x 10^4) and Thermal Parameters
of the Atoms with e.s.d. values in parenthesis

Molecule A

	x	y	z	Ueq
N_1	5040(7)	4362(3)	5532(2)	38(1)
N_2	3220	4118	4852	38(1)
C_3	2045(9)	2967(4)	4912(2)	34(1)
OC_3	94(7)	2362(3)	4373(2)	47(1)
CC_3	-713(10)	3034(4)	3696(3)	43(1)
H_1CC_3	-1358	3913	3873	47[a]
H_2CC_3	832	3107	3390	47[a]
CB_1	-3001(10)	2221(4)	3194(3)	41(1)
CB_2	-4213(12)	1124(5)	3434(3)	57(2)
HCB_2	-3583	839	3940	61[a]
CB_3	-6328(14)	427(5)	2945(4)	76(2)
HCB_3	-7203	-321	3123	78[a]
CB_4	-7205(13)	791(6)	2210(4)	68(2)
HCB_4	-8633	281	1867	83[a]
CB_5	-6019(12)	1883(7)	1963(3)	65(2)
HCB_5	-6653	2151	1453	61[a]
CB_6	3932(11)	2599(6)	2445(3)	55(2)
HCB_6	-3115	3364	2268	66[a]
C_4	3099(10)	2449(4)	5627(3)	38(1)
CC_4	2299(10)	1234(4)	5928(3)	42(1)
O_1C_4	3370(8)	846(3)	6532(2)	56(1)
[illegible]	273(8)	557(3)	5466(2)	62(1)
HO_2C_4	-693	-38	5771	166(24)[a]
C_5	5020(10)	3396(4)	5999(3)	41(1)
HC_5	6132	3366(4)	6498	47[a]
[illegible]	6469(9)	5659(4)	5703(3)	35(1)
HC[illegible]	7636	5654	6195	38[a]
[illegible]	4460(9)	6831(4)	5802(3)	36(1)
HC[illegible]	2633	6664	5533	39[a]
[illegible]	391[illegible]([illegible])	7156(3)	6605(2)	48(1)
HO[illegible]	2206	7602	6526	112(19)[a]
[illegible]	6018(9)	7898(4)	5466(3)	36(1)
HC[illegible]	4841	8608	5318	38[a]
[illegible]	8192([illegible])	8462(3)	6044(2)	44(1)
HO[illegible]	[illegible]457	8830	6520	69(14)[a]
[illegible]	[illegible]334(9)	7123(4)	4788(3)	35(1)
HC[illegible]	8906	7625	4679	40[a]
[illegible]	5640(10)	6915(5)	3978(3)	50(2)
H[illegible]	6592	6291	3625	58[a]
H[illegible]	5524	[illegible]761	3774	58[a]
[illegible]	2923([illegible])	6418(3)	3986(2)	50(1)
HO[illegible]	3300	5288	4068	138(21)[a]
[illegible]	[illegible]945(6)	5842(3)	5064(2)	39(1)

TABLE III (Cont'd)

Molecule B

	x	y	z	Ueq
N_1	8275(7)	7860(3)	8654(2)	36(1)
N_2	10226(8)	7907(3)	9307(2)	37(1)
C_3	11440(9)	6740(4)	9214(2)	37(1)
OC_3	13479(7)	6369(3)	9721(2)	49(1)
CC_3	14282(10)	7318(4)	10415(3)	43(1)
H_1CC_3	14930	8131	10251	44[a]
H_2CC_3	12726	7507	10719	44[a]
CB_1	16552(10)	6730(4)	10920(3)	42(1)
CB_2	17782(11)	5535(5)	10681(3)	56(2)
HCB_2	17162	5032	10171	57[a]
CB_3	19925(13)	5043(6)	11174(4)	72(2)
HCB_3	20812	4224	10993	76[a]
CB_4	20755(13)	5731(7)	11911(4)	73(2)
HCB_4	22178	5377	12256	79[a]
CB_5	19564(12)	6907(7)	12150(3)	65(2)
HCB_5	20183	7400	12662	69[a]
CB_6	17466(12)	7413(6)	11688(3)	57(2)
HCB_6	16628	8244	11851	59[a]
C_4	10364(10)	5926(4)	8493(3)	40(1)
CC_4	11115(11)	4590(4)	8149(3)	50(2)
O_1C_4	9948(10)	4008(4)	7530(3)	78(2)
O_2C_4	13131(9)	4089(3)	8562(2)	67(1)
HO_2C_4	13478	3253	8447	78(15)[a]
C_5	8310(10)	6710(4)	8174(3)	40(1)
HC_5	7710	6462	7691	44[a]
$C_{1'}$	6890(9)	9072(4)	8487(3)	34(1)
$HC_{1'}$	5626	8819	8018	39[a]
$C_{2'}$	8861(9)	10161(4)	8320(2)	34(1)
$HC_{2'}$	10727	10137	8564	37[a]
$O_{2'}$	9115(7)	10033(3)	7488(2)	44(1)
$HO_{2'}$	10685	10350	7306	108(19)[a]
$C_{3'}$	7401(9)	11433(4)	8641(3)	39(1)
$HC_{3'}$	8605	12207	8746	42[a]
$O_{3'}$	5140(7)	11694(3)	8083(2)	49(1)
$HO_{3'}$	5793	11463	7559	59(13)[a]
$C_{4'}$	6194(9)	11024(4)	9365(3)	40(1)
$HC_{4'}$	4665	11602	9464	44[a]
$C_{5'}$	7998(11)	11211(5)	10153(3)	52(2)
$H_1C_{5'}$	7068	10795	10535	59[a]
$H_2C_{5'}$	8209	12154	10330	59[a]
$O_{5'}$	10659(7)	10660(4)	10127(2)	53(1)
$HO_{5'}$	10587	9622	9954	92(17)[a]
$O_{1'}$	5539(7)	9609(3)	9155(2)	43(1)

Ueq is defined as one-third of the trace of the orthogonalised Uij tensor. [a] Value is the isotropic U.

TABLE IV

Hydrogen bond data

D——H······A			H——A(Å)	D···A(Å)	D—H···A(deg)	translation of A		
				intramolecular				
O_5,A[a]	HO_5,A	N_2A	1.886(2)	2.893(5)	140.5(2)[b]	x,	y,	z
O_5,B	HO_5,B	N_2B	1.918(4)	2.931(5)	161.6(2)	x,	y,	z
				intermolecular				
O_3,A	HO_3,A	O_2,B	2.003(3)	2.726(5)	132.0(2)	x,	y,	z
O_2C_4A	HO_2C_4A	O_3,A	1.735(3)	2.672(5)	157.5(5)	x-1,	y-1,	z
O_2C_4B	HO_2C_4B	O_3,B	1.830(4)	2.662(5)	162.3(3)	x+1,	y-1,	z
O_2,B	HO_2,B	O_1C_4A	2.038(4)	2.921(6)	160.0(2)	x+1,	y+1,	z
O_3,B	HO_3,B	O_1C_4A	2.024(4)	2.703(5)	125.3(2)	x,	y+1,	z

[a]The letter A or B is added to the atom label to designate the molecule to which atom belongs.

[b]The e.s.d. values on H···A and D—H···A are underestimated as the positional parameters of the H atoms were not refined. Based on past experience an uncertainty of about 0.03 Å on H···A distance and 2 to 4 degrees on D——H······A angle would be reasonable.

END

8-87

DTIC